Paving ways for personalizing drug therapy during pregnancy

A focus on the risk of drug teratogenicity

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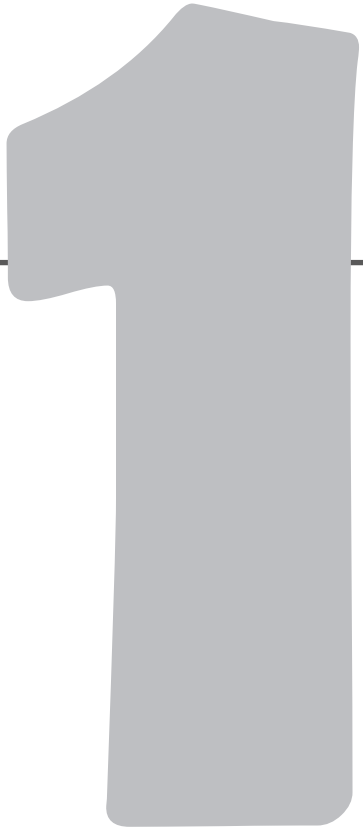
CONTENTS

chapter



General Introduction

Pregnancy is a very important period in women's life, which notably affects their emotional and physical well-being. Besides these changes, more important from a pharmacotherapeutic point of view, are the physiological changes that affect the response to drugs consumed by these women (1,2). Drug use during pregnancy has been assessed in many drug utilization studies and it was estimated that between 20 to 90% of pregnant women in developed countries were prescribed at least one drug (3–6). The actual use could be even more prevalent if we take into account the consumption of over-the-counter medicines e.g. analgesics. Some women have clear indications for taking drugs during pregnancy, which are to control their diseases and to avoid fetal complications e.g. antiepileptics, insulin, and antiretrovirals. Other women, usually with minor ailments, may choose whether to take medicines or not. This decision, however, may be affected by the unknown risk of harm to the fetus.

Drug risks during pregnancy

Some drugs are known to be teratogenic to the human fetus. A teratogenic effect is manifested by developmental toxicity in an embryo/fetus, by the induction or increase in the frequency of structural disorders (7). Drug teratogenicity was not much of a concern until the thalidomide issue came into the picture in the early 1960s. The use of thalidomide, a popular antiemetic for morning sickness, was associated with severe congenital limb anomalies also known as phocomelia. Since this event, the regulation for drug approval has been tightened, and congenital anomaly (CA) registries were set up to monitor the occurrence of CA. These registries were united in several networks, e.g. European Concerted Action on Congenital Anomalies and Twins (EUROCAT), The US National Birth Defects Prevention Network (NBDPN), and the International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR). These registries are commonly used in observational studies to detect signals of drug-induced teratogenicity. The information on drug use during pregnancy is usually derived from two types of sources, either from self-reports (interviews and questionnaires) or from data linkage with prescription databases.

The prevalence of CA is 2-3% of total births, and the highest prevalence is for congenital heart anomalies (CHA), i.e. 8 to 9 cases per 1,000 live births (8). CA can occur due to different reasons including genetic factors, maternal characteristics or environmental exposure during pregnancy (e.g. maternal age, smoking, obesity, and drugs) and also a combination of these factors (9). It is of alarming concern that about 90% of the drugs registered by the US Food and Drug Administration (FDA) between 1980 and 2012 does not have a clear teratogenicity profile in human pregnancy (10–12). There is also a significant lag time, on average 27 years, between drug discovery and the pregnancy safety data to become available (11). A Dutch study

estimated that a potentially teratogenic drug is taken in approximately five percent of all pregnancies, with doxycycline and paroxetine being the most frequently dispensed (13).

Luckily, not all drugs taken during pregnancy are deemed to cause harmful effects to the fetus. Among the determinants of fetal teratogenicity are the dose-effect relationships, the inter- and intraspecies variability in the susceptibility for teratogenic effect, and timing of drug exposure (7). First, the dose-effect relationship is important in toxicological evaluations. Nearly every drug has been tested for the 'no-effect' level, which is the level of exposure that does not promote any biological alterations that might lead to adverse effects (7). Drugs with a wide therapeutic index may provide a safe and effective therapy within a wide range of concentrations in the body (e.g. analgesics), in contrast to drugs with a narrow therapeutic index (e.g. antiepileptics). Next, not all mammalian species are equally susceptible to the teratogenic effect of a given drug. One same drug may induce different developmental disorders in different species. The timing of drug exposure, or window of susceptibility, determines the types of adverse effects on the fetus. Fetal CAs are usually associated with exposure at an early stage of pregnancy, while exposure at a later stage induces functional disorders (7).

There are several pregnancy classification systems set up by the authorities to guide clinicians in making a careful choice of drug therapy among pregnant patients, e.g. the Swedish classification, the Australian classification (the Australian Drug Evaluation Committee, ADEC) and the Teratogen Information System (TERIS) risk ratings (14). The risk classifications suggested by these systems are largely based on the data from animal studies, as human data is very limited. The limitation of animal studies is the interspecies variation in drug teratogenicity. For example, thalidomide did not pose much risk of fetal CA in mice, but it was highly teratogenic in humans, other primates, rabbits and several other species (15). Furthermore, the US FDA has removed the formerly used pregnancy risk categories (A, B, C, D, X) in the recent Pregnancy and Lactation Labeling Rule (PLLR), as these categories were not set up to represent a definitive scaled approach to the risk (16). Effective from June 2015, the PLLR includes three subsections ('Pregnancy', 'Lactation,' and 'Females and Males of Reproductive Potential), which describe risks within the real-world context of caring for pregnant women who may need medication (16,17).

Several tools are used to allow estimation of human fetal risks after the exposure to drugs. These tools include placental perfusion studies, biomarkers of fetal exposure, and epidemiological studies (18). Placental perfusion studies measure drug transport across the placenta using human placenta samples, which were taken immediately after

delivery. While this method mimics the in vivo environment in the human placenta, there are other physiological parameters that cannot be measured like protein binding and drug elimination, which might not represent the conditions during the first trimester of pregnancy (1). Biomarkers of fetal exposure, like fetal hair or meconium, were used over the last two decades to measure long-term fetal exposure to recreational drugs, alcohol and environmental chemicals (18). Finally, epidemiological studies using population-based prescription databases or birth registries can be used to estimate fetal risks. These studies (e.g. cohort and case-control studies) are prone to biases in determining the associations between drug exposure and fetal outcome, for example in the classification of exposure (19). Furthermore, the risk of a drug to the fetus can only be assessed after the drug has entered the market, and the final assessment can take up to decades. The limitations of these designs suggest the need for complementary tools or study designs to evaluate the risks of fetal drug exposure.

Pharmacogenetics and personalized drug therapy

Pharmacogenetics refers to the study of individual candidate genes as a powerful tool to help explain interindividual variability in drug response, both in therapeutic and adverse effects (20). The majority of this variability is caused by the differences in the ability of metabolic enzymes in the liver and the gastrointestinal tract to metabolize drugs. Pharmacogenetic studies seek genetic variations or polymorphisms among individuals, and measure the effect of these variations on drug response and tolerability.

Pharmacogenetics is part of personalized medicine, in which the goal is to provide a patient with the right dose of the right drug with the right duration at the right time. This model foresees drug therapy to be tailored to an individual's genetic profile. By knowing a patient's metabolic enzyme profile pertinent to the drugs taken, it will help in minimizing dosage inaccuracy and the risk of side effects. For example, pharmacogenetic algorithm including genetic variations in CYP2C9 and VKORC1 significantly improved the dose prediction for patients who required either high or low doses of warfarin. Therefore, determining the genotypes might help in faster dose optimization (21). Meanwhile, a meta-analysis study has reported the association between the long allele genotype of the serotonin transporter (SERT) with a better response to antidepressants (22). Furthermore, the determination of HLA-B*5701 polymorphism has helped in reducing the incidence of abacavir hypersensitivity syndrome (23,24).

The knowledge of pharmacogenetics has been incorporated in the healthcare system, including the drug registration regulation. Up until 2015, approximately

15% of drugs licensed by the European Medicines Agency (EMA) (25) and at least 130 drugs according to the US FDA contain pharmacogenetic data in the product information (26). These data include the direct impact of the genotypes on the dosage recommendation and the important role of pharmacogenetics in optimizing benefits and reducing risks of drugs. The EMA has also produced a series of guidelines in the development of pharmacogenetic methods and assays in clinical development of drugs, including the draft of 'Good Pharmacogenomics Practice' (27). In the Netherlands, the genotype-guided dosing recommendations for at least 53 drugs have been issued since 2006. These recommendations are incorporated in the national computerized system for drug prescribing, dispensing and medication surveillance (28,29). Although the translation of pharmacogenetics into clinical practice is not as fast as expected, pharmacogenetics seems to be a promising advance in drug therapy (30).

Why would pregnant women benefit from personalized drug therapy?

Personalized drug therapy is aimed to benefit all patient populations, including that of pregnant women. Specific reasons to consider this concept among this population of interest include:

- Pregnant women are more vulnerable to changes in drug efficacy and safety, because of both physiological changes during pregnancy and pharmacogenetic variations **(Figure 1)** (31,32). These changes might either lead to underdosing or overdosing of a drug, which poses the risk of inefficacy or toxicity.

- The right use of drugs during pregnancy affects not only the mother but also the unborn child. Some drugs target the unborn child specifically (e.g. antiretrovirals or corticosteroids for lung maturation). These drugs therefore need to be transported through the placenta in optimal concentrations. The transport of teratogenic drugs, on the other hand, should be as low as possible.

Personalized drug therapy in obstetrics and maternal-fetal medicine has been proposed by clinicians and researchers since 2010 (20,33-36), but not much studies were done to explore this further. For instance, it was shown that polymorphisms in maternal/fetal metabolic enzymes seemed to play a role in variations of corticosteroid and nifedipine pharmacokinetics administered before labor (37,38). One of the challenges in prenatal pharmacogenomics research is to obtain fetal and placental samples in a specific gestational period of interest, as the physiological condition and the expression of relevant enzymes/proteins changes throughout gestation.

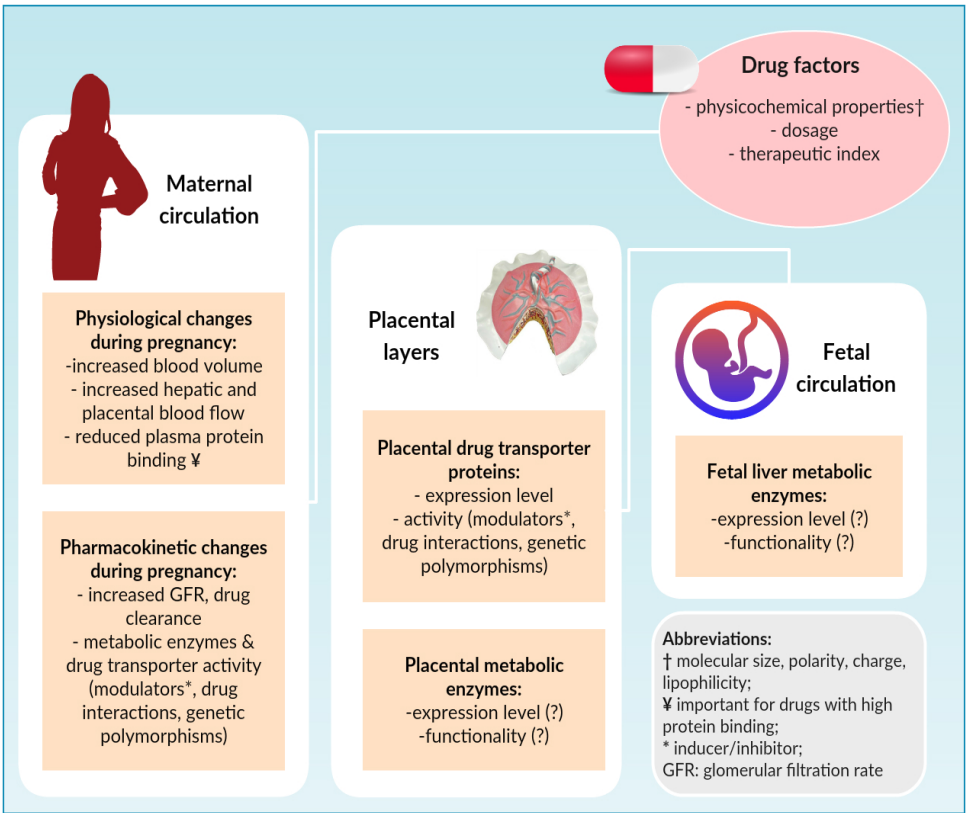


Figure 1: Schematic diagram of the parameters to be taken into account in the extent of fetal drug exposure following maternal drug use during pregnancy

Potential parameters in predicting fetal drug exposure and fetal risk

In this thesis, we investigated concepts that are relevant to personalized drug therapy in pregnancy: 1) the role of placental transporter proteins in fetal drug exposure, and 2) pharmacogenetic predictors associated with the risk of drug teratogenicity.

The role of placental transporter proteins in fetal drug exposure

Transporter proteins play a major role in drug disposition. Transporters expressed in the placenta act as the gatekeepers for many compounds, including drugs, passing through the placental layer **(Figure 2)**. The most studied transporter is P-glycoprotein (P-gp) which is encoded by the *ABCB1* gene. Several modulators and genetic polymorphisms of the *ABCB1* gene have been associated with changes in P-gp expression and activity in the placenta (30). These changes may alter the amount of drugs transported into the fetal circulation, thus modulating fetal drug exposure and fetal teratogenic risk (40).

Another factor that may modulate fetal drug exposure is the effect of drug-drug interaction. Pregnant women might use more than one drug during the course of treatment and the use of an inhibitor or inducer of the placental transporters may affect substrate drug transport into the fetal circulation (41-43). With regard to this, we explored the role of drug-drug interactions mediated by P-gp and other placental transporters on the risk of CA, as a proxy to fetal drug exposure.

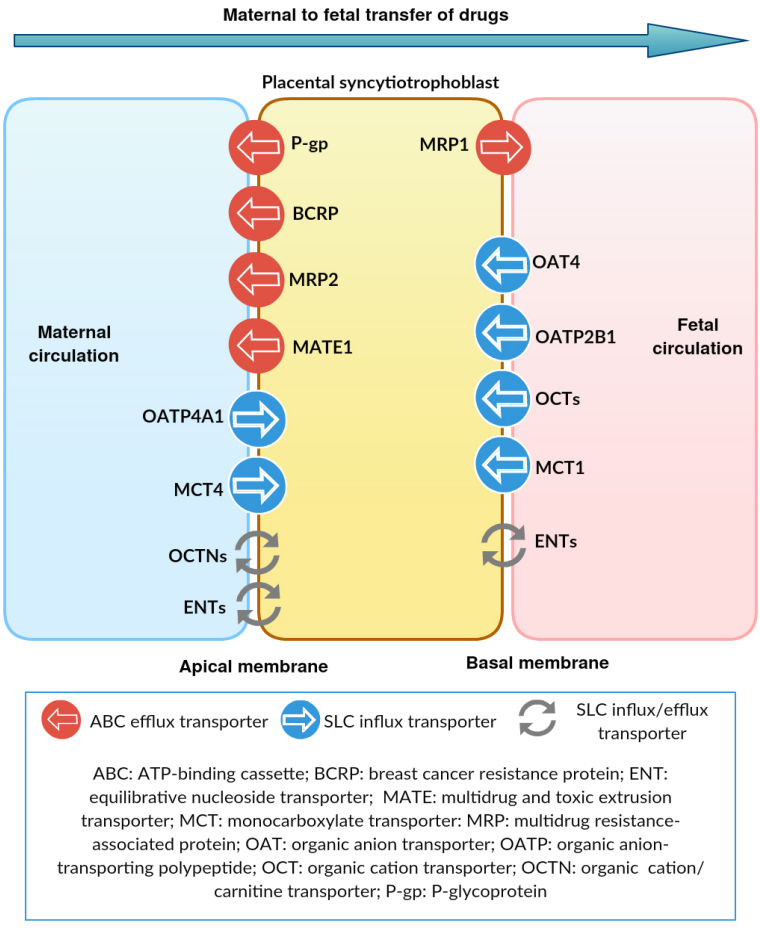


Figure 2: Drug transporter proteins expressed at the apical and basal membrane of the placental syncytiotrophoblast cells

Pharmacogenetic predictors associated with the risk of drug teratogenicity

One of the most important concerns in fetal adverse events is the risk of CA. A much discussed issue in prenatal drug therapy in the past decade is the increased risk of fetal congenital heart anomalies (CHA) following maternal use of antidepressants, especially serotonin reuptake inhibitors (SRIs). Meta-analyses have reported a 40% increase in the risk of cardiovascular malformation after maternal paroxetine use in the first trimester of pregnancy (44-46). Approximately 3 to 5% of pregnant women use antidepressants, especially SRIs, making this issue a public health concern (3,47-49). The US FDA has therefore issued a warning in 2005 with the advice to discontinue paroxetine treatment either before or within weeks of becoming pregnant (50,51).

The safety of SRI use during pregnancy has been broadly studied since the FDA warning, mainly via retrospective epidemiological studies. Most studies do take into account several confounders, for instance, maternal age at delivery, parity and smoking or alcohol intake in early pregnancy. Based on current evidence, many factors need to be considered before advising women to discontinue antidepressant treatment when they are pregnant, including stage of pregnancy, maternal medication history, and the risk of relapse after discontinuation (52,53).

With regard to the association between prenatal exposure to SRIs and CHA, we determined possible pharmacogenetic predictors relevant to the pharmacokinetics and pharmacodynamics of SRIs. We then explored whether genetic variations in these predictors may help in determining which child is more at risk of CHA. The result may hopefully contribute to a better risk stratification among women taking SRIs during the first trimester of pregnancy.

Thesis objectives

As mentioned above, we are aiming to pave the way for personalized drug therapy during pregnancy. The objectives of this thesis can be listed as follows:

1) To evaluate the role of placental transporter proteins in modulating fetal drug exposure

2) To determine the knowledge of pharmacogenetics among formerly pregnant women, and to assess their attitude towards the use of pharmacogenetics in future drug prescribing

3) To explore the pharmacogenetic predictors associated with the risk of congenital heart anomalies in children exposed to serotonin reuptake inhibitors *in utero*

Thesis outline

Part A focuses on the role of placental transporter proteins in fetal drug exposure. In **chapter 2** and **chapter 3**, we determine whether the inhibition of these transporters may modulate fetal drug exposure to the fetus. The outcome of CA was used as a proxy for fetal drug exposure. We performed pharmacoepidemiology studies using data from the population-based congenital anomaly registry (EUROCAT Northern Netherlands) and the population-based prescription database of the University of Groningen (the IADB.nl). Details of both databases are described in Box 1 and 2. **Chapter 4** is a review article which contains the latest knowledge about polymorphisms of placental transporter proteins and their effects on protein expression in the placenta.

Part B introduces the concept of pharmacogenetics in drug use during pregnancy. In **chapter 5**, we describe the knowledge of a population of formerly pregnant women regarding pharmacogenetics, and we assess their attitude towards the implementation of pharmacogenetics in their future drug therapy. The next chapters focus on the role of pharmacogenetics in drug-induced CA, specifically on the association between the use of SRIs and the risk of CHA. **Chapter 6** gives a complete overview of the polymorphisms in the genes that are associated with the pharmacokinetics of SRIs and the mechanisms of action of this drug class. **Chapter 7** describes a gene-environment interaction study that explores the potential pharmacogenetic predictors of the risk of CHA associated with prenatal exposure to SRIs.

Finally, **Chapter 8** provides an overview of the studies and general discussion on the topic, including future perspectives and recommendations for future research.

Box 1: EUROCAT Northern Netherlands (NNL) - Birth defect registry

EUROCAT Northern Netherlands (NNL) was established in 1981 as a regional registry of EUROCAT, a network of population-based registries of congenital anomalies (CA) in Europe (54). EUROCAT focuses on the prevalence, primary prevention and prenatal diagnosis of CA. EUROCAT NNL registers CA cases in three Dutch provinces (Groningen, Friesland and Drenthe), and is funded by the Dutch Ministry of Health, Welfare and Sport.

Notification of children and fetuses with congenital anomalies is voluntary. Registry personnel are actively involved in case ascertainment, using multiple sources such as obstetric records, hospital administration data, and pathology records (54). All types of births are registered: live births, stillbirths, spontaneous abortions and terminations of pregnancy. Anomalies are coded with the International Classification of Diseases (ICD)-9 for births prior to 2002, and ICD-10 for births from 2002 onwards and are classified according to the EUROCAT Subgroups of Congenital Anomalies (55). Annually, EUROCAT NNL monitors between 16,000-19,000 births in the region.

Children with CA can be registered in Eurocat NNL until they reach the age of ten years. Since 1989, parents have to give consent for registration of their child. Among eligible children with anomalies, the participation rate has been stable at approximately 80% for many years. Starting from 1997, after consenting to registration, parents receive a questionnaire asking about their health, life style, use of medication, fertility, and pregnancy; another 80% completes the questionnaire. After maternal consent, the information on drug use from three months before conception until delivery was collected from the pharmacy records and was later verified by telephone interviews. The actual use of both prescribed drugs, as well as over-the-counter (OTC) drugs, was completely registered, including the daily dose, prescription status and period of use during pregnancy. The drugs are coded using the Anatomical Therapeutic Chemical (ATC) classification system (56).

Box 2: IADB.nl prescription database

The IADB.nl is a longitudinal, population-based prescription database in the Netherlands. It was started in 1998 as the InterAction Database, a collaborative project between community pharmacists and the University of Groningen.

Data are collected from at least 55 community pharmacies, in the three Northern provinces (Groningen, Friesland and Drenthe), and cover an estimated population of 600,000 people. The prescription rates in this population have been found to be representative for the whole population in the Netherlands. IADB.nl records the dispensing data of all delivered drugs for all patients, as prescribed by both general practitioners and specialists in the outpatient clinics. Drugs dispensed in hospitals or over the counter medicines are not recorded. Data include the name of the prescribed drug, date of dispensing, quantity dispensed, dose regimen, prescribing physician and ATC codes (57).

To study drug use during pregnancy, a subset of data is extracted from IADB.nl, the so called Pregnancy IADB. A woman is assumed to be the mother of a child, if she is 15–50 years older than the child and they are both registered to the same address. With this methodology, approximately 65% of the children in the main IADB.nl database could be linked to their mothers, and validation of the identified mothers showed 99% accuracy (58). This linkage, however, lacks the information about the length of pregnancy.

REFERENCES

1. Anderson GD. Pregnancy-induced changes in pharmacokinetics: A mechanistic-based approach. Clin Pharmacokinet. 2005;44(10):989–1008.

2. Gaohua L, Abduljalil K, Jamei M, Johnson TN, Rostami-Hodjegan A. A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4. Br J Clin Pharmacol. 2012;74(5):873–85.

3. Andrade SE, Gurwitz JH, Davis RL, Chan KA, Finkelstein J a, Fortman K, et al. Prescription drug use in pregnancy. Am J Obstet Gynecol. 2004 Aug;191(2):398–407.

4. Mitchell AA, Gilboa SM, Werler MM, Kelley KE, Louik C, Hernández-Díaz S. Medication use during pregnancy, with particular focus on prescription drugs: 1976-2008. Am J Obstet Gynecol. 2011;205(1):51.e1-51.e8.

5. Daw JR, Hanley GE, Greyson DL, Morgan SG. Prescription drug use during pregnancy in developed countries : a systematic review. Pharmacoepidemiol Drug Saf. 2011;20:895–902.

6. Cleary BJ, Butt H, Strawbridge JD, Gallagher PJ, Fahey T, Murphy DJ. Medication use in early pregnancy-prevalence and determinants of use in a prospective cohort of women. Pharmacoepidemiol Drug Saf. 2010;19:408–17.

7. Peters P, Miller RK, Schaefer C. General commentary on drug therapy and drug risks in pregnancy. In: Schaefer C, Peters P, Miller RK, editors. Drugs during pregnancy and lactation. 3rd ed. Munich: Elsevier B.V.; 2014. p. 1–23.

8. Van Der Linde D, Konings EEM, Slager MA, Witsenburg M, Helbing WA, Takkenberg JJM, et al. Birth prevalence of congenital heart disease worldwide: A systematic review and meta-analysis. J Am Coll Cardiol. 2011;58(21):2241–7.

9. Zhu H, Kartiko S, Finnell RH. Importance of gene - environment interactions in the etiology of selected birth defects. Clin Genet. 2009;75(5):409–23.

10. Lo WY, Friedman JM. Teratogenicity of recently introduced medications in human pregnancy. Obstet Gynecol. 2002;100(3):465–73.

11. Adam MP, Polifka JE, Friedman JM. Evolving knowledge of the teratogenicity of medications in human pregnancy. Am J Med Genet C Semin Med Genet. 2011 Aug 15;157C(3):175–82.

12. Mazer-Amirshahi M, Samiee-Zafarghandy S, Gray G, Van Den Anker JN. Trends in pregnancy labeling and data quality for US-approved pharmaceuticals. Am J Obstet Gynecol. 2014;211(6):690.e1-690.e11.

13. Zomerdijk I, Ruiter R, Houweling L, Herings R, Straus S, Stricker B. Dispensing of potentially teratogenic drugs before conception and during pregnancy: A population-based study. BJOG An Int J Obstet Gynaecol. 2015;122:1119–29.

14. Schirm E, Meijer WM, Tobi H, De Jong-Van Den Berg LTW. Drug use by pregnant women and comparable non-pregnant women in the Netherlands with reference to the Australian classification system. Eur J Obstet Gynecol Reprod Biol. 2004;114(2):182–8.

15. Vargesson N. Thalidomide-induced teratogenesis: History and mechanisms. Birth Defects Res Part C Embryo Today Rev. 2015;105:140–56.

16. Greene MF. FDA drug labeling for pregnancy and lactation drug safety monitoring systems. Semin Perinatol. 2015;39(7):520–3.

17. US FDA Pregnancy and Lactation Labeling (Drugs) Final Rule. [Internet]. Available from: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/Labeling/ucm093307.htm. Last accessed 22 December 2016.

18. Etwel F, Hutson JR, Madadi P, Gareri J, Koren G. Fetal and perinatal exposure to drugs and chemicals: novel biomarkers of risk. Annu Rev Pharmacol Toxicol. 2014;54:295–315.

19. Grzeskowiak LE, Gilbert AL, Morrison JL. Exposed or not exposed? Exploring exposure classification in studies using administrative data to investigate outcomes following medication use during pregnancy. Eur J Clin Pharmacol. 2012;68(5):459–67.

20. Haas DM. Pharmacogenetics and individualizing drug treatment during pregnancy. Pharmacogenomics. 2014;15(1):69–78.

21. International Warfarin Pharmacogenetic Consortium. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med. 2009;306(8):753–64.

22. Porcelli S, Fabbri C, Serretti A. Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with antidepressant efficacy. Eur Neuropsychopharmacol. 2012 Apr;22(4):239–58.

23. Rauch A, Nolan D, Martin A, McKinnon E, Almeida C, Mallal S. Prospective genetic screening decreases the incidence of abacavir hypersensitivity reactions in the Western Australian HIV cohort study. Clin Infect Dis. 2006;43(1):99–102.

24. Hetherington S, Hughes AR, Mosteller M, Shortino D, Baker KL, Spreen W, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. Lancet. 2002;359:1121–2.

25. Ehmann F, Caneva L, Prasad K, Paulmichl M, Maliepaard M, Llerena A, et al. Pharmacogenomic information in drug labels: European Medicines Agency perspective. Pharmacogenomics J. 2015;15:201–10.

26. US Food and Drug Administration. Table of pharmacogenomic biomarkers in drug labeling [Internet]. 2016. Available from: http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm. Last accessed 30 December 2016.

27. European Medicines Agency. Guideline on good pharmacogenomic practice [Internet]. 2016. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000411.jsp&mid=WC0b01ac058002958e. Last accessed 22 December 2016.

28. Swen JJ, Wilting I, de Goede AL, Grandia L, Mulder H, Touw DJ, et al. Pharmacogenetics: from bench to byte. Clin Pharmacol Ther. 2008;83(5):781–7.

29. Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, et al. Pharmacogenetics: from bench to byte-an update of guidelines. Clin Pharmacol Ther. 2011;89(5):662–73.

30. Wilffert B, Swen J, Mulder H, Touw D, Maitland-Van Der Zee A-H, Deneer V. From evidence based medicine to mechanism based medicine. Reviewing the role of pharmacogenetics. Int J Clin Pharm. 2013;35(3):369–75.

31. Isoherranen N, Thummel KE. Drug metabolism and transport during pregnancy : How does drug disposition change during

pregnancy and what are the mechanisms that cause such changes ? Drug Metab Dispos. 2013;41:256–62.

32. Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H. Anatomical, physiological and metabolic changes with gestational age during normal pregnancy. Clin Pharmacokinet. 2012;51(6):365–96.

33. Blumenfeld YJ, Reynolds-May MF, Altman RB, El-Sayed YY. Maternal-fetal and neonatal pharmacogenomics: a review of current literature. J Perinatol. 2010;30(9):571–9.

34. Haas DM. Obstetric therapeutics-How pharmacogenetics may inform drug therapy for pregnant women in the future. Obstet Gynecol Surv. 2013;68(9):650–4.

35. Quinney SK, Patil AS, Flockhart DA. Is personalized medicine achievable in obstetrics? Semin Perinatol. 2014;38(8):534–40.

36. Dorfman EH, Cheng EY, Hebert MF, Thummel KE, Burke W. Prenatal pharmacogenomics: a promising area for research. Pharmacogenomics J. 2016;(May):1–2.

37. Haas DM, Lehmann AS, Skaar T, Philips S, McCormick CL, Beagle K, et al. The impact of drug metabolizing enzyme polymorphisms on outcomes after antenatal corticosteroid use. Am J Obstet Gynecol. 2012;206(5):e17-24.

38. Haas DM, Quinney SK, Clay JM, Renbarger JL, Hebert MF, Clark S, et al. Nifedipine pharmacokinetics are influenced by CYP3A5 genotype when used as a preterm labor tocolytic. Am J Perinatol. 2013;30(4):275–82.

39. Hutson JR, Garcia-Bournissen F, Davis A, Koren G. The human placental perfusion model: a systematic review and development of a model to predict in vivo transfer of therapeutic drugs. Clin Pharmacol Ther. 2011;90(1):67–76.

40. Pollex EK, Hutson JR. Genetic polymorphisms in placental transporters: implications for fetal drug exposure to oral antidiabetic agents. Expert Opin Drug Metab Toxicol. 2011;7(3):325–39.

41. Endres CJ, Hsiao P, Chung FS, Unadkat JD. The role of transporters in drug interactions. Eur J Pharm Sci. 2006 Apr;27(5):501–17.

42. Rubinchik-Stern M, Eyal S. Drug Interactions at the Human Placenta: What is the Evidence? Front Pharmacol. 2012;3:1–7.

43. Muller F, Fromm MF. Transporter-mediated drug–drug interactions. Pharmacogenomics. 2011;12(7):1017–37.

44. Grigoriadis S, VonderPorten EH, Mamisashvili L, Roerecke M, Rehm J, Dennis C-L, et al. Antidepressant exposure during pregnancy and congenital malformations: is there an association? A systematic review and meta-analysis of the best evidence. J Clin Psychiatry. 2013 Apr;74(4):e293-308.

45. Wurst KE, Poole C, Ephross SA, Olshan AF. First trimester paroxetine use and the prevalence of congenital, specifically cardiac, defects: a meta-analysis of epidemiological studies. Birth defects Res A, Clin Mol Teratol. 2010;88(3):159–70.

46. Myles N, Newall H, Ward H, Large M. Systematic meta-analysis of individual selective serotonin reuptake inhibitor medications and congenital malformations. Aust N Z J Psychiatry. 2013;47(11):1002–12.

47. Colvin L, Slack-Smith L, Stanley FJ, Bower C. Dispensing patterns and pregnancy outcomes for women dispensed selective serotonin reuptake inhibitors in pregnancy. Birth Defects Res Part A - Clin Mol Teratol. 2011;91(3):142–52.

48. Charlton R, Jordan S, Pierini A, Garne E, Neville A, Hansen A, et al. Selective

serotonin reuptake inhibitor prescribing before, during and after pregnancy: a population-based study in six European regions. BJOG. 2014 Oct 28;doi: 10.1111/1471-0528.13143.

49. Alwan S, Reefhuis J, Rasmussen SA, Friedman JM. Patterns of antidepressant medication use among pregnant women in a United States population. J Clin Pharmacol. 2011;51(2):264–70.

50. Petersen I, Gilbert RE, Evans SJW, Man SL, Nazareth I. Pregnancy as a major determinant for discontinuation of antidepressants: An analysis of data from The Health Improvement Network. J Clin Psychiatry. 2011;72(7):979–85.

51. Jimenez-Solem E, Andersen JT, Petersen M, Broedbaek K, Andersen NL, Torp-Pedersen C, et al. Prevalence of Antidepressant Use during Pregnancy in Denmark, a Nation-Wide Cohort Study. PLoS One. 2013;8(4):1–8.

52. Howard LM, Megnin-Viggars O, Symington I, Pilling S. Antenatal and postnatal mental health: summary of updated NICE guidance. BMJ. 2014;349:g7394.

53. Petersen I, Evans SJ, Gilbert R, Marston L, Nazareth I. Selective serotonin reuptake inhibitors and congenital Heart anomalies: Comparative cohort studies of women treated before and during pregnancy and their children. J Clin Psychiatry. 2016;77(1):e36–42.

54. Greenlees R, Neville A, Addor MC, Amar E, Arriola L, Bakker M, et al. Paper 6: EUROCAT member registries: Organization and activities. Birth Defects Res Part A - Clin Mol Teratol. 2011;91(SUPPL. 1):S51–100.

55. EUROCAT Guide 1.3 and reference documents Instructions for the Registration and Surveillance of Congenital Anomalies (2005) [Internet]. Available from: http://www.eurocat-network.eu/content/EUROCAT-Guide-1.3.pdf. Last accessed 22 December 2016.

56. WHO Collaborating Centre for Drug Statistics Methodology. ATC/DDD Index 2017. [Internet]. 2017. Available from: https://www.whocc.no/atc_ddd_index/. Last accessed 22 December 2016.

57. Visser ST, Schuiling-Veninga CC, Bos JH, de Jong-van den Berg LTW, Postma MJ. The population-based prescription database IADB.nl : its development, usefulness in outcomes research and challenges. Expert Rev Pharmacoeconomics Outcome Res. 2013;13(3):285–92.

58. Schirm E, Tobi H, de Jong-van den Berg LTW. Identifying parents in pharmacy data: a tool for the continuous monitoring of drug exposure to unborn children. J Clin Epidemiol. 2004 Jul;57(7):737–41.

part

THE ROLE OF TRANSPORTER PROTEINS IN DRUG-INDUCED BIRTH DEFECTS

- P-glycoprotein-mediated drug interactions in pregnancy & the changes on the risk of congenital anomalies: A case-reference study
- Maternal use of drug substrates of placental transporters and the effect of transporter-mediated drug interactions on the risk of congenital anomalies
- Pharmacogenetics of drug-induced birth defects: the role of polymorphisms of placental transporter proteins

chapter

P-glycoprotein-mediated drug interactions in pregnancy and the changes in the risk of congenital anomalies

A case-reference study

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ABSTRACT

Background: Drug use in pregnancy is very common but may cause harm to the fetus. The teratogenic effect of a drug is partly dependent on the drug level in the fetal circulation, which is associated with the transport across the placenta. Many drugs are substrates of P-glycoprotein (P- gp), an efflux transporter that acts as a protective barrier for the fetus. We aim to identify whether drug interactions associated with P-gp promote any changes in fetal drug exposure, as measured by the risk of having children with congenital anomalies.

Methods: In this study, cases (N = 4634) were mothers of children with congenital anomalies registered in the EUROCAT Northern Netherlands registry, and the reference population were mothers of children (N = 25,126) from a drug prescription database (IADB.nl).

Results: Drugs that are associated with P-gp transport were commonly used in pregnancy in cases (10%) and population (12%). Several drug classes, which are substrates for P-gp, were shown to have a higher user rate in mothers of cases with specific anomalies. The use of this subset of drugs in combination with other P-gp substrates increased the risk for specific anomalies (odds ratio [OR] 4.17, 95% CI 1.75–9.91), and the addition of inhibitors further increased the risk (OR 13.03, 95% CI 3.37–50.42). The same pattern of risk increment was observed when the drugs were analyzed separately according to substrate specificity.

Conclusions: The use of drugs associated with P-gp transport was common during pregnancy. For several drug classes associated with specific anomalies, P-gp-mediated drug interactions are associated with an increased risk for those specific anomalies.

INTRODUCTION

Drug use in pregnancy is inevitable in some conditions, particularly in chronic diseases such as diabetes, depression and epilepsy. Drugs are also often prescribed to alleviate the symptoms associated with pregnancy or to fight infections (1). In fact, between 27 and 93% of pregnant women in several developed countries were prescribed drugs, excluding vitamins and minerals, during pregnancy (2). Moreover, the prescription rate during the first trimester is approximately 40%, and nearly 3% of the pregnant women were prescribed drugs suspected to be teratogenic to the fetus (1,3).

The risk of drug teratogenicity is dependent on the degree of fetal exposure, which is regulated partly by the transporter proteins in the placenta (4). One of the most abundant transporter proteins is P-glycoprotein (P-gp), an efflux transporter expressed in the maternal-facing surface of the placental tissue (5,6). P-gp acts as a barrier to preventing some potentially harmful drugs from entering the fetal circulation, and its function can be modulated by inhibitors and inducers (5,7). Many drugs are known to be substrates of P-gp, some of which may also inhibit or induce this transporter. The use of P-gp inhibitors or inducers in combination with a substrate, referred to as 'P-gp-mediated drug interactions', may affect the P-gp barrier function in the placenta and subsequently fetal exposure to this substrate. For substrates that are teratogenic, the changes in transport and thereby of fetal exposure may be reflected in a changed risk of fetal congenital anomalies. Despite the importance of drug interactions in the placenta for fetal outcome, far too little attention has been given to this issue.

P-gp-mediated drug interactions have been previously explored in *in vitro* and *in vivo* studies (8,9). P-gp inhibitors increase the placental transfer of P-gp substrates in placental samples of late pregnancy (10); however, the level of P-gp expression changes during pregnancy and the effect on fetal drug exposure during the early stage of pregnancy is not really known. There is still no established method to pursue this, particularly because of the difficulty in obtaining placental samples in early pregnancy.

The aim of this study was to explore the role of P-gp on fetal drug exposure in the first trimester of pregnancy. Applying a case-reference study design, we used the outcome of congenital anomalies as a proxy for fetal drug exposure. The first objective was to describe the pattern of use of drugs associated with P-gp transport during the first trimester of pregnancy, while the second objective was to determine the effect of P-gp-mediated drug interactions on the risk of congenital anomalies in exposed children.

METHODS

Case selection

Cases were selected among the mothers of children registered by EUROCAT Northern Netherlands (NNL), a population-based registry covering the northern provinces of The Netherlands (Groningen, Friesland and Drenthe). The registry includes fetuses or children, up to the age of 10 years, with congenital anomalies diagnosed before or after birth. All mothers of the children in this study gave consent to register their child in EUROCAT. EUROCAT records detailed information on the sociodemographic and lifestyle characteristics of the mothers, obtained through questionnaires. With the consent of the mothers, the information on drug use from 3 months before conception until delivery was collected from the pharmacy records and was later verified by telephone interviews.

Among eligible children born in the study period (1997–2013), 81.6% of parents gave consent to register their child in EUROCAT, and 80.2% returned the questionnaire and gave consent to retrieve their pharmacy data (personal communication). The actual use of both prescribed drugs, as well as over- the-counter (OTC) drugs, was completely registered, including the prescription status of any OTCs used (whether they were prescribed or not). Other details on drug use included, among others, the Anatomical Therapeutic Chemical (ATC) codes and the period of use during pregnancy. The congenital anomalies were classified based on the EUROCAT Subgroup of Congenital Anomalies (11) and the International Classification of Diseases (ICD) coding system, ninth revision (ICD9) until 2001 and tenth revision (ICD10) from 2002 onwards. Only major anomalies were included in this study. The EUROCAT Subgroups of Congenital Anomalies (ICD9, ICD10) were anomalies of the nervous system (740–742, Q00–Q07), eye, ear, face and neck (743–744, Q10–Q18), heart (745, 746, 7470–7474, Q20–Q26), respiratory (748, Q30–Q34), orofacial clefts (7490–7492, Q35–Q37), digestive system (750–751, 7566, Q38–Q45, Q790), urinary (753, 75,672, 75,261, Q60–Q64, Q794), and genital (7520–7524, 75,260, 75,262, 7527–7529, Q50–Q52, Q54–Q56). Two subgroups of anomalies were classified according to the ICD10, i.e. (ICD9, ICD10) anomalies of the musculoskeletal system (754, 7566–7567, Q65–Q66, Q790–Q795) and limb (755, Q69–Q74).

A total of 6934 children with congenital anomalies were born between 1 January 1997 and 31 December 2013. Overall, 885 children with genetic and chromosomal abnormalities were excluded because these anomalies were not likely to be related to drug use. We selected 5268 of the cases in which mothers had a history of drug use during pregnancy to match the reference population from a prescription database.

Mothers with a previous history of children with anomalies were excluded in order to avoid selection bias in drug selection and prescribing, which led to recruitment of 4634 cases.

Reference Population Sampling

Reference data were obtained from the IADB.nl, a longitudinal, population-based prescription database in The Netherlands. The data were collected from 55 community pharmacies, including Groningen, Friesland and Drenthe, and covered an estimated population of 600,000 people. The prescription rates in this population have been found to be representative for the whole population in The Netherlands in comparison with data from insurance companies throughout the whole country (12). Pharmacies periodically update the data of all delivered drugs for all patients, as prescribed by both general practitioners and specialists in the outpatient clinics. Data include the name of the prescribed drug, date of dispensing, quantity dispensed, dose regimen, prescribing physician and ATC codes (12).

A pregnancy database, Pregnancy IADB, was obtained from a large mother–child subset extracted from IADB.nl. All liveborn children born between 1997 and 2013 were selected from this database. For each child, the female person aged 15–50 years older than the child and registered to the same address code is considered to be the mother. If there is another female person in that age range and with the same address code, the child will be excluded. Approximately 65% of children in the main IADB.nl database are linked to their mother, and validation of the identified mothers showed 99% accuracy (13).

The gestational period was determined from the theoretical conception date by subtracting the date of birth of the children with 273 days (gestational period of 9 months). Since the gestation period for twin and triplet pregnancies is likely to be shorter than singleton pregnancies, twin and triplet pregnancies were excluded. These pregnancies were identified based on the number of children with the same date of birth who were linked to the same mother. If more than one child was linked, the mother was excluded.

All registered pregnancies in the Pregnancy IADB, regardless of the number of pregnancies per mother, were the source population (N = 38,129). We then selected only the first registered pregnancy for each mother as the reference population (N = 25,126). The first known pregnancy for each mother was selected to overcome misclassification bias in maternal drug use since drug selection may be influenced by the previous pregnancy outcome. Ethical approval was not necessary for cases and population sampling since only anonymous data were used.

Exposure variables

The user rates of drugs associated with P-gp transport during pregnancy were described in the source population to give a general overview of the prevalence at the population level. The P-gp drugs were identified through a literature review and classified according to substrate affinity, consisting of P-gp substrate, substrate/inhibitor, substrate/inducer, inhibitor, inducer, inhibitor/inducer and substrate/inhibitor/ inducer (5,14–16). This classification was determined by the results from *in vivo* studies and if not available, *in vitro* studies. More details on the list of all identified drugs are available in **Appendix 1.1**. Drugs that are substrates/inducers are substrates when used alone but act as inducers to P-gp in the presence of another substrate. The same applies for substrates/inhibitors, which may inhibit the transport of another P-gp substrate when both are used concurrently. Inhibitors/inducers and substrates/inhibitors/inducers may become either one of these classes depending on substrate selectivity to P-gp in comparison with the other interacting drug.

The user rates include OTC drugs. The Dutch OTC drug list was obtained from the Medicines Evaluation Board of The Netherlands. The period of exposure was restricted to the period of organogenesis, i.e. the first trimester. Some drugs that are dispensed before the conception date may also be continually used during the first trimester, therefore we also included the preconception period. Children were considered to be exposed to the drugs if the period of the drugs dispensed/used was within 90 days before conception and the first 90 days of pregnancy.

To determine the effect of P-gp-mediated drug interactions on the risk of congenital anomalies, only drugs that showed associations with teratogenicity were selected. To find potential teratogenic drugs, we first grouped the drugs into drug classes according to their pharmacological action. Second, we calculated the user rates of these drug classes for all EUROCAT Subgroups of Congenital Anomalies. Drug classes with significantly higher user rates in specific subgroups of congenital anomalies were selected as 'drugs with associations'. Information on the use of OTCs is not complete in the Pregnancy IADB; therefore, for a better comparison with cases, the use of OTC drugs was disregarded in this analysis. Throughout the study period, four drugs underwent changes in prescription regulation in The Netherlands (OTC to prescription drug, and vice versa), i.e. ranitidine, domperidone, omeprazole and pantoprazole. We included the exposure of these drugs among the case group, only when they were prescribed, so that the exposure status was comparable to the reference population.

Exposure was defined as mothers presented with P-gp- mediated drug interaction patterns, which included the combination of drugs transported by P-gp with another substrate and/or inducer and/or inhibitor.

Statistical analysis

The analysis strategy for drug interaction patterns was conducted based on a priori hypothesis in which the distribution of types of medication used would differ between cases and the reference population, with an increased use in cases indicating a possible interaction. We also stratified the drug interaction analysis into substrate specificity (substrates, substrates/inhibitors) to observe whether the risk on anomalies was different for each group of drugs.

The Chi-square test was used in the comparison between the number of users in cases of specific anomalies and other anomalies. The number of cases of specific anomalies was then compared with the number of the reference population presented with the same pattern of P-gp-mediated drug interactions by calculating the odds ratio (OR) and 95% confidence interval (CI). An OR of more than 1 indicated an increased risk for congenital anomalies with certain interaction patterns. The results were considered as statistically significant at $p \leq 0.05$. Since some drugs are classified as 'P-gp substrate' and some as 'P-gp substrate/ inhibitor', the analysis was conducted separately for each group. Analyses were performed using PASW Statistics, version 22 (IBM Corporation, Armonk, NY, USA).

RESULTS

Characteristics of the children born to case mothers are presented in **Table 1**. The majority of children were live born, and the most common types of anomalies were the heart and musculoskeletal anomalies, which were each present in more than 20% of the children. Maternal age at delivery was comparable between cases, source and reference populations, i.e. 30.3 years for case mothers, 30.0 years for mothers from the source population and 29.4 years for mothers from the reference population. A lower number of children were born in the last 5 years of the study period (2009–2013) in the source population (18%) and reference population (15%) compared with cases (22%) because children were only registered in the IADB.nl once they received any prescription drug.

Table 1: Characteristics of children born to case mothers (N=4,634)

Characteristics		N	%
Gender	Boy	2617	56.5
	Girl	2011	43.4
	Missing	6	0.1
Type of birth	Live birth	4361	94.1
	Termination of pregnancy	184	4.0
	Stillbirth	58	1.3
	Miscarriage (>24 weeks)	31	0.7
Types of anomalies*	Heart	1244	26.8
	Musculoskeletal	1054	22.7
	Digestive system	594	12.8
	Urinary	507	10.9
	Oro-facial clefts	436	9.4
	Genital	406	8.8
	Nervous system	347	7.5
	Limb	342	7.4
	Eye, ear, face & neck	152	3.3
	Respiratory	84	1.8

*Percentage does not add to 100 as cases with multiple anomalies are counted more than once.
mparison with the reference population

User Rates of Drugs Associated with P-Glycoprotein (P-gp) During Pregnancy

The use of drugs associated with P-gp transport is fairly common during pregnancy. Of 105 P-gp substrates identified in the literature, including OTC drugs, 44 were used by at least one case mother, while 66 were prescribed to at least one mother in the source population. The list of drugs and number of users among the cases and the reference population is available in the **Appendix 1.1**.

Furthermore, one or more of these drugs were used by 17.7% (n = 820) of case mothers and 14.5% (n = 5543) of mothers in the source population, suggesting that drugs associated with P-gp transport are widely used, even in pregnancy. The Pregnancy IADB also records the OTCs but only when the drugs are prescribed by physicians; therefore, the number of users in the source population is likely to be underestimated.

Among case mothers, 10.4% (n = 481) had used at least one drug associated with P-gp transport, and 12.0% (n = 3022) of mothers in the reference population were prescribed these drugs. The user rates and prescription rates of these drugs, according to substrate specificity, are shown in **Figure 1**. Doxycycline, a P-gp substrate, was the most commonly used or prescribed drug (case mothers, n = 102 [2.2%]; reference mothers, n = 595 [2.4%]), followed by omeprazole, a P-gp substrate/inhibitor (case mothers, n = 41 [0.9%]; reference mothers, n = 294 [1.2%]). For drug groups indicated for chronic diseases, the user rate of selective serotonin reuptake inhibitors (SSRIs) was the highest [case mothers, n = 86 (1.9%); reference mothers, n = 607 (2.4%)] compared with other antidepressants, antipsychotics and antiepileptics.

In the selection of drugs with associations, most OTCs were excluded, as explained in the Methods section. Among cases, 13 drugs were identified that were used in a higher percentage in cases with specific anomalies compared with cases with other anomalies (**Table 2**). Cimetidine and ranitidine (H2-receptor antagonists) were shown to have an association with heart anomalies (p = 0.037), omeprazole and pantoprazole (proton pump inhibitors [PPIs]) with genital anomalies (p = 0.046), morphine with respiratory anomalies (p = 0.018), and antipsychotics (haloperidol, quetiapine and risperidone) with musculoskeletal anomalies (p = 0.018). Drugs that were found to have associations with specific anomalies were grouped as 'drugs with associations' and were used in the risk estimation analysis for P-gp-mediated drug interactions in congenital anomalies. The association between SSRIs (paroxetine, sertraline, fluoxetine, fluvoxamine, citalopram) and nervous system anomalies nearly reached statistical significance (p = 0. 054), but due to previous warnings of teratogenicity, this drug class was also classified as 'drugs with associations'.

Additional analysis on the risk of specific anomalies with the respective drug class was carried out by comparing the user rates in case mothers and mothers in the reference population. Increases in ORs were found for most of the drug classes with specific anomalies, but failed to reach statistical significance.

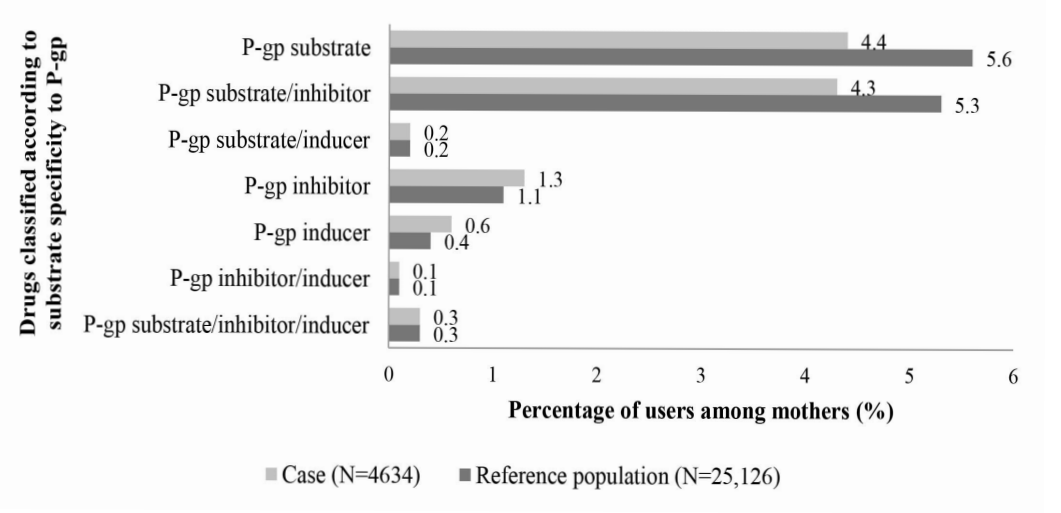

Figure 1: The user rates (in case mothers) and prescription rates (in mothers of the reference population) of drugs associated with P-gp transport. One mother may be counted more than once.

The classifications of drugs according to P-gp substrate specificity and drugs with at least one user in both cases and reference population: **P-gp substrate** (cimetidine, ranitidine, domperidone, propranolol, betamethasone, hydrocortisone, triamcinolone, doxycycline, tetracycline, sumatriptan, lamotrigine, levetiracetam, risperidone, clomipramine, nortriptyline, citalopram, venlafaxine, fexofenadine); **P-gp substrate/inhibitor** (omeprazole, pantoprazole, simvastatin, atorvastatin, clarithromycin, azithromycin, ketoconazole, itraconazole, cyclosporine, haloperidol, quetiapine, amitriptyline, fluoxetine, paroxetine, sertraline, fluvoxamine, terfenadine); **P-gp substrate/inducer** (dexamethasone, morphine, phenytoin, carbamazepine); **P-gp inhibitor** (progesterone, duloxetine, mefloquine); **P-gp inducer** (insulin); **P-gp inhibitor/inducer** (bromocriptine, midazolam); **P-gp substrate/ inhibitor/inducer** (erythromycin, diltiazem).

Table 2: The number of users of drug/drug classes associated with specific congenital anomalies, and the risk determination of these anomalies from the comparison with the reference population

Drug/drug classes	Types of specific anomalies	Number of case users (%)		*p* value	Number of users in reference population (N=25,126), (%)	OR (95% CI)	*p* value
		Within specific anomalies	*Within all other anomalies (N=4,634 - n_s)*				
H2-receptor antagonist (cimetidine, ranitidine)	Heart (n_s=1244)	5 (0.4)	3 (0.1)	0.037†	161 (0.64)	0.63 (0.26-1.53)	0.3
Proton pump inhibitors (omeprazole, pantoprazole)	Genital (n_s=406)	9 (2. 2)	52 (1.2)	0.046	327 (1.3)	1.72 (0.88-3.36)	0.11
Morphine	Respiratory (n_s=84)	1 (1.2)	7 (0.2)	0.018†	3 (0.01)	100.9 (10.39-979.94)	0.13†
Antipsychotics (haloperidol, quetiapine, risperidone)	Musculoskeletal (n_s=1,054)	5 (0.5)	3 (0.1)	0.018†	57 (0.23)	2.1 (0.84-5.24)	0.1
Selective serotonin reuptake inhibitors (fluoxetine, citalopram, paroxetine, sertraline, fluvoxamine)	Nervous system (n_s=347)	11 (3.2)	74 (1.7)	0.054*	576 (2.3)	1.4 (0.76-2.56)	0.28

n_s: number of cases of respective type of anomalies

†Fisher's exact test

*Although not significant, SSRIs will be included in further analysis due to previous warnings of teratogenicity

P-gp-Mediated Drug Interactions and the Risk of Congenital Anomalies

The risk of congenital anomalies was found to be affected by P-gp-mediated drug interactions (**Figure 2**). For all P-gp substrates, including drugs without associations, we could not show an effect of the combination with any inhibitor or inducer on the risk for overall anomalies compared with the use of the substrate alone (OR 0.81, 95% CI 0.52–1.27, p = 0.4). However, the risk for specific anomalies was significantly increased to nearly threefold in mothers using any of the 13 drugs with associations in combination with other substrates (OR 4.17, 95% CI 1.75–9.91, p = 0.003). The risk was further augmented up to sixfold with the addition of inhibitors (OR 13.03, 95% CI 3.37–50.42, p = 0.003). The risk estimations for individual drugs with associations are available in **Appendix 1.2**.

The same pattern of risk increment was observed when analyses were conducted separately for substrates and substrate/inhibitor groups within the drugs with associations. For substrates (cimetidine, ranitidine, risperidone, citalopram), herein referred to as 'drug', the use of 'drug + substrate(s)' significantly increased the risk of specific anomalies compared with its use alone (OR 7.64, 95% CI 1.61–36.33, p = 0.023). The combination of 'drug + substrate(s) + inhibitor(s)' showed a slight increase in risk but failed to reach statistical significance, probably because of the low number of exposed cases. For substrates/inhibitors (omeprazole, pantoprazole, haloperidol, quetiapine, fluoxetine, paroxetine, sertraline, fluvoxamine), the combination of 'drug + substrate(s)' significantly increased the risk of specific anomalies by approximately threefold (OR 3.21, 95% CI 1.12–9.23, p = 0.04). The addition of inhibitor(s) further increased the risk up to tenfold, but with a large confidence interval (OR 13.33, 95% CI 2.58–68.96, p = 0.017).

Morphine was the only substrate/inducer used in both cases and the reference population; one user among cases and three users in the reference population. These numbers impede further analysis of drug interactions for morphine. However, this drug was included in the analysis where all drugs with associations were combined.

Study or Subgroup	Case Events	Case Total	Reference Events	Reference Total	Odds Ratio M-H, Fixed, 95% CI
A) All P-gp substrates + inhibitor/inducer	24	195	207	1397	0.81 [0.51, 1.27]
B) Drugs with assoc. + substrate	6	22	78	763	3.29 [1.25, 8.66]
C) Drugs with assoc. + substrate + inhibitor	3	19	19	704	6.76 [1.82, 25.17]
D) Drugs with assoc. (S) + substrate	1	4	10	79	2.30 [0.22, 24.32]
E) Drugs with assoc. (S) + substrate + inhibitor	1	4	2	71	11.50 [0.80, 165.11]
F) Drugs with assoc. (S/I) + substrate	5	18	68	682	3.47 [1.20, 10.04]
G) Drugs with assoc. (S/I) + substrate + inhibitor	2	15	17	631	5.56 [1.16, 26.57]

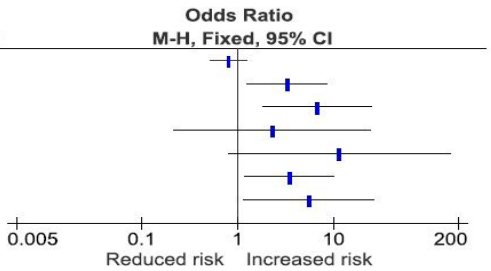

Figure 2: The risk estimation of overall and specific anomalies with several patterns of P-gp-mediated drug interactions

The reference for the OR calculation is the use of drug substrates alone for each subgroup.

A) showed no increased risk of overall anomalies associated with drug interactions in 41 P-gp substrates, p=0.4 (the list of the drugs can be found in **Figure 1** legend);

B) and **C)** showed the risk changes for specific anomalies (heart, genital, respiratory, musculoskeletal and nervous system) in 13 drugs previously associated with these anomalies (cimetidine, ranitidine, omeprazole, pantoprazole, morphine, haloperidol, risperidone, quetiapine, citalopram, fluoxetine, paroxetine, sertraline, fluvoxamine), both with p=0.003;

D) and **E)** showed the risk changes for specific anomalies (heart, musculoskeletal and nervous system) in P-gp substrates previously associated with these anomalies (cimetidine, ranitidine, risperidone, citalopram), p=0.023 and 0.16, respectively;

F) and **G)** showed the risk changes for specific anomalies (genital, musculoskeletal and nervous system) in P-gp substrates/ inhibitors previously associated with these anomalies (omeprazole, pantoprazole, haloperidol, quetiapine, fluoxetine, paroxetine, sertraline, fluvoxamine), p=0.04 and 0.017, respectively. S, substrates; S/I, substrates/inhibitors; *$p<0.05$ (Fisher's exact test)

DISCUSSION

Our study was the first to describe the pattern of use of drugs associated with P-gp transport in the first trimester of pregnancy. We also found that P-gp-mediated drug interactions have an effect on the risk of congenital anomalies, as a proxy for fetal exposure. It has already been shown that these interactions affect drug pharmacokinetics and the pharmacodynamic effects of multiple drugs, mostly based nn the interactions occurring in the small intestine, liver and kidney (see review by Akamine et al. (16)). Since P-gp is also substantially expressed in the placenta, the P-gp inhibition and induction is expected to play a role in determining fetal drug exposure. P-gp inhibition is expected to lead to a higher amount of drugs transferred into the fetal circulation, which can potentially harm the fetus. The pattern of risk increment between 'drug + substrate(s)' and 'drug + substrate(s) + inhibitor(s)' was the same for P-gp substrates and substrates/inhibitors, although we found a large CI due to a limited number of exposed mothers.

Several preclinical studies have demonstrated the role of P-gp inhibition in the drug transport mechanism in the placenta, using placental samples after delivery (approximately 37–42 weeks of gestation). In studies using the dually perfused human placental model, the maternal-to-fetal transport of saquinavir, indinavir and lopinavir was shown to increase with P-gp inhibition (17,18). The transfer of paclitaxel, methadone and talinolol were also affected by the drug interactions mediated by P-gp (19–21). In order to evaluate the role of P-gp-mediated drug interactions in the risk of congenital anomalies, it is important that the P-gp level in preclinical studies is comparable to that observed in the early stages of pregnancy. As the expression level of P-gp is known to decrease when pregnancy advances (22, 23), it may be expected that the results of these preclinical studies may not fully demonstrate the degree of P-gp inhibition and the effect on fetal drug exposure in the first trimester of pregnancy. The placental expression of P-gp itself is also subject to interindividual variation, in which the expression level may differ among mothers (19). However, the correlation between expression level and activity of the placental P-gp also remains unclear, leading to the need for different methods in order to evaluate the net effect of placental drug transfer (19).

Apart from P-gp transport, there are other factors that may contribute to the net concentration of a drug in the placenta. These factors include changes in placental permeability which may readily alter maternal-to-fetal drug disposition (24). Increased permeability of the placenta is caused by a reduction in placental thickness, as well as increased surface area and placental blood flow. Another factor is the drug interactions mediated by the metabolic enzymes, the cytochrome (CYP) 450.

P-gp and CYP450 enzymes were shown to share broad substrate specificity (16,25). In this study, we assume that, at least from 2002, the clinically significant CYP-mediated drug interactions had already been avoided during prescribing and dispensing by the software-supported medications surveillance commonly used in The Netherlands (26). Furthermore, the metabolic enzymes expressed in the placenta seemed to add minor contributions to fetal drug transfer (10). The last factor was the genetic variability of the genes encoding for P-gp and CYP450 relevant to the pharmacokinetics of each drug investigated. Genotype-dependent drug interactions can also cause variations in the drug's efficacy and toxicity profile (27,28).

Since this study aims to explore the role of P-gp in fetal drug exposure using a technical approach, the list of drugs tested in this study was based on *in vivo* and *in vitro* studies with regard to their substrate specificity to P-gp transport. A drug identified in an *in vitro* study is not necessarily of clinical importance, and extrapolating from animal evidence to humans is not always simple. To improve the quality of results in future studies, it is suggested to focus on specific drug classes with documented clinical evidence on P-gp transport activity.

Although the proportion of drug transferred across the placenta is considered minimal, any change in the placental barrier mechanism may cause significant changes in fetal drug concentration. These changes can be detrimental to the fetus, especially if the drugs have a small therapeutic index, are teratogenic, or can potentially affect the normal physiological transport of nutrients to the fetus (29).

Strengths and limitations

Our study was the first to report on the occurrence of P-gp- mediated drug interactions in pregnant women and the effect on the fetus. Drug use and the period of use are well-documented in both databases used in this study, and the cases and population sampling were carried out in the same time period. Moreover, the prescription records in the IADB.nl are virtually complete due to the high patient–pharmacy commitment in The Netherlands (12).

In the determination of the 'drugs with associations', we did not find the previously reported associations between drug or drug groups with specific types of anomalies, for example antiepileptics and neural tube defects (30). There are several reasons for this. First, our study was not set up to find these associations because we focused on P-gp-mediated drug interactions and therefore we used the user rates from cases with all other anomalies within our case group as a reference group. It is a convenient method to quickly identify potentially harmful drugs and then continue with the next analysis to identify whether drug interactions may change the risk. In contrast,

in most pharmacovigilance studies, the aim is to find a signal of teratogenicity of a drug, and the major malformation rates are usually calculated by using total birth as a denominator. Second, our study might be underpowered to detect previously reported associations.

One of our limitations was selection bias in the reference population since only children who had received prescription drugs were registered in the IADB.nl. In the IADB.nl, only 65% of children in the population could be linked to their mother. The most important reasons for missing the linkage were that the mother had a separate address registration number, the mother was registered with another pharmacy in the town, or the mother did not live at the same address (13). We do not have information on the non-linked children; however, since linkage is based on birth date and address rather than health profiles, the linkage may have been random and is unlikely to have substantially influenced exposure and outcome. Although the Pregnancy IADB can only link a child to a mother if they had taken prescription drugs, it is well-known that approximately 80% of children, before the age of 2 years, have been prescribed at least one medication that was dispensed from the pharmacy (31). We therefore assumed that the reference population was comparable to the unselected group.

There was also a potential classification bias in the exposure period of drugs in the reference population as the gestational period of the pregnancies in the reference population was based on estimation (assumption of 293 days of gestation). Furthermore, exposure definition differs between cases and the reference population, which is based on actual use and prescriptions, respectively. Actual exposure in the reference population is expected to be lower than observed, which may have led to an underestimation of the observed increased risk. IADB.nl only registered the live births, therefore we cannot include stillborn children and pregnancies that underwent miscarriage or termination. There is a possibility that the risk estimations for congenital anomalies in the results are overestimated if the exposure rate in these children/pregnancies are higher compared with liveborn children. However, due to a low rate of stillbirths, miscarriages and terminations of pregnancy in the population-based statistics (0.2–0.4 % for stillbirths (32)), we expect the change to be very minimal. A similar assumption applies to children in the Pregnancy IADB who might also have congenital anomalies. The rate of congenital anomalies is approximately 3 % of the total number of births, therefore these numbers are not expected to cause significant changes in risk estimation (33).

Confounding by indication was also assumed to have minor implications on the results. This type of confounding refers to those situations in which the indication for treatment acts as a confounder, in this case the disease influences the exposure to

P-gp substrates and acts as a risk factor for congenital anomalies. In this study, the exposure variable consists of a broad range of drugs, each with specific indications. One of the known risk factors for congenital anomalies is maternal diabetes mellitus (34), and insulin is one of the drugs associated with P-gp transport; however, insulin is an inducer to this transporter, not a substrate, therefore it is not included in the analysis for the effect of P-gp inhibition on the risk of congenital anomalies.

There are also other confounding factors that we were not able to address, such as maternal smoking behavior, alcohol consumption and other risk factors for congenital anomalies. Therefore, our results warrant further epidemiological studies, preferably with a higher number of exposed cases and focusing on exposure to selected teratogenic drugs in specific congenital anomalies in combination with potent inhibitors or inducers.

CONCLUSIONS

The use of drugs associated with P-gp transport was common in pregnancy, and some mothers had to use more than one of these drugs. For several drug classes that were associated with specific anomalies, P-gp-mediated drug interactions were found to increase the risk for those anomalies. Apart from other factors that may affect fetal drug exposure, for these drug classes, P-gp plays a clinically relevant role in limiting drug exposure, as previously suggested in preclinical studies. The absolute risk of such interactions may be modest, but at the P-gp level it showed a significant increment in the risk of congenital anomalies in drugs suspected to be teratogenic. Moreover, this knowledge may assist drug individualization in pregnancy, especially in chronic diseases.

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REFERENCES

1. Bakker MK, Jentink J, Vroom F, Van Den Berg PB, De Walle HEK, De Jong-Van Den Berg LTW. Drug prescription patterns before, during and after pregnancy for chronic, occasional and pregnancy-related drugs in the Netherlands. BJOG. 2006;113:559–68.

2. Daw JR, Hanley GE, Greyson DL, Morgan SG. Prescription drug use during pregnancy in developed countries: a systematic re- view. Pharmacoepidemiol Drug Saf. 2011;20:895–902.

3. Andrade SE, Gurwitz JH, Davis RL, Chan KA, Finkelstein JA, Fortman K, et al. Prescription drug use in pregnancy. Am J Obstet Gynecol. 2004;191:398–407.

4. Staud F, Cerveny L, Ceckova M. Pharmacotherapy in pregnancy: effect of ABC and SLC transporters on drug transport across the placenta and fetal drug exposure. J Drug Target. 2012;20:736–63.

5. Schinkel AH, Jonker JW. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Adv Drug Deliv Rev. 2003;55:3–29.

6. Ceckova-Novotna M, Pavek P, Staud F. P-glycoprotein in the placenta: expression, localization, regulation and function. Reprod Toxicol. 2006;22:400–10.

7. Zhou SF. Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. Xenobiotica. 2008;38:802–32.

8. Balayssac D, Authier N, Cayre A, Coudore F. Does inhibition of P-glycoprotein lead to drug-drug interactions? Toxicol Lett. 2005;156:319–29.

9. Aszalos A. Drug-drug interactions affected by the transporter protein, P-glycoprotein (ABCB1, MDR1) I. Preclinical aspects. Drug Discov Today. 2007;12:833–7.

10. Rubinchik-Stern M, Eyal S. Drug interactions at the human placenta: what is the evidence? Front Pharmacol. 2012;3:1–7.

11. European Surveillance of Congenital Anomalies (EUROCAT). EUROCAT guide 1.3 and reference documents. Instructions for the registration and surveillance of congenital anomalies. 2005. Available at: http://www.eurocat-network.eu/content/EUROCAT-Guide-1.3.pdf. Last accessed 27 Oct 2014.

12. Visser ST, Schuiling-Veninga CC, Bos JH, de Jong-van den Berg LTW, Postma MJ. The population-based prescription database IADB.nl: its development, usefulness in outcomes research and challenges. Expert Rev Pharmacoecon Outcomes Res. 2013;13:285–92.

13. Schirm E, Tobi H, de Jong-van den Berg LTW. Identifying parents in pharmacy data: a tool for the continuous monitoring of drug exposure to unborn children. J Clin Epidemiol. 2004;57:737–41.

14. Endres CJ, Hsiao P, Chung FS, Unadkat JD. The role of trans- porters in drug interactions. Eur J Pharm Sci. 2006;27:501–17.

15. Hodges LM, Markova SM, Chinn LW, Gow JM, Kroetz DL, Klein TE, et al. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). Pharmacogenet Genomics. 2011;21:152–61.

16. Akamine Y, Yasui-Furukori N, Ieiri I, Uno T. Psychotropic drug- drug interactions involving P-glycoprotein. CNS Drugs. 2012;26:959–73.

17. Molsa M, Heikkinen T, Hakkola J, Hakala K, Wallerman O, Wadelius M, et al. Functional role of P-glycoprotein in the human blood-placental barrier. Clin Pharmacol Ther. 2005;78:123–31.

18. Ceccaldi P-F, Gavard L, Mandelbrot L, Rey E, Farinotti R, Treluyer J-M, et al. Functional role of p-glycoprotein and binding

protein effect on the placental transfer of lopinavir/ritonavir in the ex vivo human perfusion model. Obstet Gynecol Int. 2009;2009:726593.

19. Nanovskaya T, Nekhayeva I, Karunaratne N, Audus K, Hankins GD, Ahmed MS. Role of P-glycoprotein in transplacental transfer of methadone. Biochem Pharmacol. 2005;69:1869–78.

20. May K, Minarikova V, Linnemann K, Zygmunt M, Kroemer HK, Fusch C, et al. Role of the multidrug transporter proteins ABCB1 and ABCC2 in the diaplacental transport of talinolol in the term human placenta. Drug Metab Dispos. 2008;36:740–4.

21. Hemauer SJ, Patrikeeva SL, Nanovskaya TN, Hankins GD, Ahmed MS. Opiates inhibit paclitaxel uptake by P-glycoprotein in preparations of human placental inside-out vesicles. Biochem Pharmacol. 2009;78:1272–8.

22. Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, Gibb W. Expression of the multidrug resistance P-glycoprotein (ABCB1 glycoprotein) in the human placenta decreases with advancing gestation. Placenta. 2006;27:602–9.

23. Gil S, Saura R, Forestier F, Farinotti R. P-glycoprotein expression of the human placenta during pregnancy. Placenta. 2005;26:268–70.

24. Unadkat JD, Dahlin A, Vijay S. Placental drug transporters. Curr Drug Metab. 2004;5:125–31.

25. Kim RB. Drugs as P-glycoprotein substrates, inhibitors, and in- ducers. Drug Metab Rev. 2002;34:47–54.

26. Van Roon EN, Flikweert S, Le Comte M, Langendijk PNJ, Kwee-Zuiderwijk WJM, Smits P, et al. Clinical relevance of drug-drug interactions: a structured assessment procedure. Drug Saf. 2005;28:1131–9.

27. Muller F, Fromm MF. Transporter-mediated drug–drug interactions. Pharmacogenomics. 2011;12:1017–37.

28. Blumenfeld YJ, Reynolds-May MF, Altman RB, El-Sayed YY. Maternal-fetal and neonatal pharmacogenomics: a review of current literature. J Perinatol. 2010;30:571–9.

29. Prouillac C, Lecoeur S. The role of the placenta in fetal exposure to xenobiotics: importance of membrane transporters and human models for transfer studies. Drug Metab Dispos. 2010;38:1623–35.

30. Tomson T, Battino D. Teratogenic effects of antiepileptic drugs. Lancet Neurol. 2012;11:803–13.

31. Schirm E, van den Berg P, Gebben H, Sauer P, De Jong-van den Berg L. Drug use of children in the community assessed through pharmacy dispensing data. Br J Clin Pharmacol. 2000;50:473–8.

32. Cousens S, Blencowe H, Stanton C, Chou D, Ahmed S, Steinhardt L, et al. National, regional, and worldwide estimates of stillbirth rates in 2009 with trends since 1995: a systematic analysis. Lancet. 2011;377:1319–30

33. European Surveillance of Congenital Anomalies (EUROCAT). EUROCAT prevalence data tables. 2015. Available at: http:// www.eurocat-network.eu/ ACCESSPREVALENCEDATA/ PrevalenceTables. Last accessed 12 Dec 2014

34. Ornoy A, Reece EA, Pavlinkova G, Kappen C, Miller RK. Effect of maternal diabetes on the embryo, fetus, and children: con- genital anomalies, genetic and epigenetic changes and develop- mental outcomes. Birth Defects Res C Embryo Today. 2015;105:53–72.

chapter 3

Maternal use of drug substrates of placental transporters and the effect of transporter-mediated drug interactions on the risk of congenital anomalies

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ABSTRACT

Background: A number of transporter proteins are expressed in the placenta, and they facilitate the placental transfer of drugs. The inhibition of P-glycoprotein (P-gp) was previously found to be associated with an increase in the risk of congenital anomalies caused by drug substrates of this transporter. We now explore the role of other placental transporter proteins.

Methods: A population-based case-referent study was performed using cases with congenital anomalies (N=5,131) from EUROCAT Northern Netherlands, a registry of congenital anomalies. The referent population (N=31,055) was selected from the pregnancy IADB.nl, a pharmacy prescription database.

Results: Ten placental transporters known to have comparable expression levels in the placenta to that of P-gp, were selected in this study. In total, 147 drugs were identified to be substrates, inhibitors or inducers, of these transporters. Fifty-eight of these drugs were used by at least one mother in our cases or referent population, and 28 were used in both. The highest user rate was observed for the substrates of multidrug resistance-associated protein 1, mainly folic acid (6% of cases, 8% of referents), and breast cancer resistance protein, mainly nitrofurantoin (2.3% of cases, 2.9% of referents). In contrast to P-gp, drug interactions involving substrates of these transporters did not have a significant effect on the risk of congenital anomalies.

Conclusions: Some of the drugs which are substrates or inhibitors of placental transporters were commonly used during pregnancy. No significant effect of transporter inhibition was found on fetal drug exposure, possibly due to a limited number of exposures.

INTRODUCTION

Drug use in pregnancy raises many concerns about the risk of harmful effects on the fetus while the use of these medications is inevitable to control certain medical conditions. The potential harmful effects of drugs on the fetus are dependent upon, among others, the concentration of drug that reaches the fetal circulation, a factor which is partly modulated by placental transport of drugs.

A number of transporter proteins are expressed in the placenta to facilitate the transport of biological substances to and from the fetus, including a subset of drugs (1-4). This transport can be modulated by interactions with other drugs transported by the same transporter. These interactions may result in changes in substrate concentration in the fetal circulation without affecting the maternal blood or plasma concentration of substrate drugs (5). The effect of drug interactions mediated by P-glycoprotein (P-gp), the most studied transporter protein, on fetal drug exposure has been described earlier (6-11). From our previous study, the risk of specific fetal congenital anomalies was increased when the mothers used P-gp substrates in combination with other substrates or inhibitors (11).

To date, the effects of drug interactions mediated by other placental transporters were observed only in *in vitro* studies (5,12,13). Therefore, we aimed to describe the user rates of drugs transported by placental transporters during the first trimester of pregnancy using population-based databases. The second objective was to investigate the effect of drug interactions mediated by these transporters on fetal drug exposure by assessing the changes in the risk of congenital anomalies.

METHODS

Cases sampling

Cases were selected from EUROCAT Northern Netherlands (NNL), a population-based registry for children with congenital anomalies born in the Northern provinces of the Netherlands. EUROCAT NNL registers fetuses or children with major congenital anomalies diagnosed before or after birth, and up to 10 years old, upon consent for their parents. The information available in the database includes sociodemographic characteristics of the parents and lifestyle during pregnancy. The information on drug intake was obtained from pharmacy records and then verified by a telephone interview with the mothers. Drug use was coded using the Anatomical Therapeutic Chemical (ATC) codes, and noted either as prescribed or over-the-counter (OTC).

Cases of major and minor congenital anomalies were classified according to EUROCAT Subgroup of Congenital Anomalies version 2012 (14), the International Classification of Diseases (ICD) coding system 9th revision for cases registered until 2001, and ICD 10th revision for cases registered from 2002 onwards. We included only major anomalies: anomalies of the nervous system, eye, ear, face & neck, heart, respiratory, oro-facial clefts, digestive system, urinary, genital, and limb (**Appendix 2.1**).

There are 6,059 cases, excluding cases with chromosomal anomalies, born between January 1, 1997 and December 31, 2013 and registered in EUROCAT NNL in March 2015. This number includes only those children whose mothers had a history of medication use at any time during pregnancy in order to match with the referent population of drug users from the prescription database. We excluded 572 cases with genetic disorders, i.e. microdeletion and monogenic disorders. To avoid selection bias in drug prescribing, we included only the first malformed child or pregnancy, which resulted in 5,131 cases.

Referent population sampling

The referent population was selected from IADB.nl, a population-based prescription database in the Netherlands. IADB.nl holds the pharmacy data from about 600,000 people, covering several parts of the country, mostly in the Northern provinces. The data were collected from 60 participating community pharmacies, and the prescription rates of the IADB.nl population were found to be representative for the population in the Netherlands as a whole (15). Prescriptions registered in the database were prescribed by general practitioners or specialists, which include the name of the drugs, the dispensing date, the ATC codes, dose and quantity dispensed (15).

For studies on drug use in pregnancy, Pregnancy IADB.nl was constructed based on the main IADB.nl, with linkage of prescription data of mother and child based on the coding of home address. The date of conception was determined by assuming a gestational age 273 days before the date of birth of the linked child. Twin or triplet pregnancies were excluded because the gestation period is likely to be shorter than singleton pregnancies. Details of the linkage and their validation are as reported earlier (16). We selected children born within the same time period as the cases, whose mothers were registered with complete information on drug use. We then selected only the first registered pregnancy (N=31,311) to avoid selection bias in the drugs prescribed, since the drug selection may be influenced by the outcome of a previous pregnancy. Since EUROCAT NNL and Pregnancy IADB.nl cover a similar geographical area, it is possible that some children were registered in both databases.

We therefore excluded 256 children (0.8% out of 31,311) from the Pregnancy IADB.nl, because the birth dates of the mothers and children, and the gender of the child matched with the children in EUROCAT NNL.

Drug exposure definitions

Selection of placental transporters proteins and drug substrates

Our previous study showed that P-gp inhibition was associated with an increased risk of congenital anomalies caused by drug substrates of P-gp, suggesting the importance of this transporter in fetal drug exposure (11). Therefore, in this study, we included all other placental transporters that have mRNA expression level in the placenta at least comparable to that of P-gp, which is 0.0255 as a ratio of the expression of peptidylprolyl isomerase (PPIA) mRNA, a housekeeping gene (17-19). From these transporters, protein expression of breast cancer resistance protein (BCRP), organic cation transporter 3 (OCT3), organic anion transporter 4 (OAT4) and organic anion transporting polypeptide 2B1 (OATP2B1) was detected in the first trimester placenta so far (20-22). The expression of BCRP increases throughout gestation but later decreases within the third trimester, while OCT3 is moderately increased throughout gestation (21,23,24). As for OAT4 and OATP2B1, not much has been reported on the changes of placental expression of these transporters during pregnancy. For the other transporters no protein expression data are available.

The selection of drug substrates of the transporters is based on review articles that report the results from *in vitro* and *in vivo* studies (5,25-29). The articles were searched in PubMed using combinations of these keywords: "placenta", "drug transporters", "drug substrate", "drug inhibitor", "Breast Cancer Resistance Protein", "ABCG2", "Multidrug resistance-associated proteins", "MRP1", "Organic anion transporters", "OAT4", "OATP2B1", "OCT3", "Monocarboxylate transporters", "MCT1", "MCT4", "MCT8", "MCT10", "equilibrative nucleoside transporters", and "ENT1". These drugs were classified according to substrate affinity to each placental transporter, including substrate, substrate/inhibitor, inhibitor, substrate/inducer and inducer, as previously done (11).

User rates of drugs associated with placental transporters

For the first objective, we described the user rates of drugs associated with transporter proteins between cases and referent population. User rates were defined as the self-reported use of (in cases), or the pharmacy dispensing of (in referent population) the selected drugs, from three months before the estimated conception date through the first three months of pregnancy. We included the preconception period because the drugs may be continually used during the first trimester of pregnancy, for the referent population. The use of OTC drugs was disregarded because the Pregnancy IADB.nl does not register the use of these drugs. However, prescribing regulations for ranitidine, ibuprofen and aspirin changed in the Netherlands during the study period, as reported by the Medicines Evaluation Board of the Netherlands. Therefore, we only included the use of prescribed ranitidine, ibuprofen and aspirin among cases. Since folic acid is usually taken as an OTC medication, its use was also included only when it was prescribed.

Drug interactions and the risk of congenital anomalies

For the second objective, we explored the effect of drug-drug interactions involving placental transporter proteins on the risk of fetal teratogenicity. We assessed the risk of overall congenital anomalies with the use of all substrates of each placental transporter. The analyses were performed for individual transporters, as fetal drug exposure may depend upon whether the influx or efflux has changed, and the localization of the transporters. Since BCRP is known to be involved in a vectorial transport with OATP2B1 (1), we also determined the effect of drug interactions involving the substrates of both placental transporters.

Statistical analysis

The user rates of the selected drugs were calculated by using the total number of cases or the population as the denominator. For the drug interaction study, binary logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals for the risk of anomalies with each drug interaction pattern. Analyses were performed using PSAW Statistics, Version 22 (IBM Corporation, Armonk, NY, USA).

RESULTS

Selection of placental transporters and drug substrates

Our study focused on placental transporters that have comparable expression levels to P-gp in the placenta, and we have identified ten eligible placental transporters: BCRP and multidrug resistance-associated protein 1 (MRP1) as efflux transporters, while OCT3, equilibrative nucleoside transporter (ENT1), OAT4, OATP2B1 and monocarboxylate transporters 1, 4, 8 and 10 (MCT1, MCT4, MCT8, MCT10) as influx transporters. The localization of these transporters in placental tissue and the direction of substrate transport are as depicted in **Figure 1**.

From the literature, 168 drugs are classified to be associated with the transport of these placental transporters, either as substrates, inducers or inhibitors. We excluded 21 of these drugs because they are either experimental or veterinary drugs, or without ATC codes. There were a total of 147 drugs selected to investigate the first objective: 53 drugs associated with BCRP transport, 45 drugs with OCT3, 29 drugs with OAT4, 28 drugs with MRP1, 24 drugs with OATP2B1, 8 drugs with ENT1, 10 drugs with MCT1, 6 drugs with MCT4, and 3 drugs with both MTC8 and MCT10 (one drug may be associated with the transport of more than one transporter). The list of all placental transporters included in this study and their respective drug substrates, inducers and inhibitors can be found in **Appendix 2.2**.

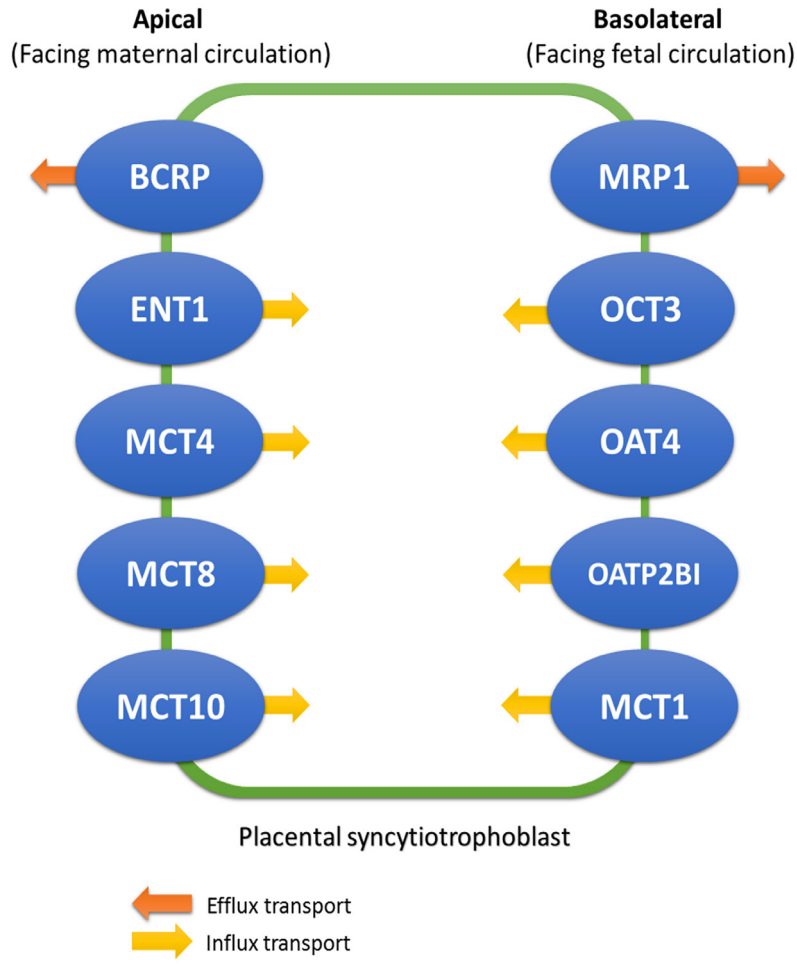

Figure 1: Placental transporter proteins are expressed on either side of the placenta. Five transporter proteins are expressed in the apical (maternal-facing) layer of placental cells, which include breast cancer resistance protein (BCRP), equilibrative nucleoside transporter (ENT), monocarboxylate transporter 4, 8, 10 (MCT4, MCT8, MCT10). Another five are expressed in the basolateral (fetal-facing) layer of placental cells, including multidrug resistance-associated protein (MRP), organic cation transporter 3 (OCT3), organic anion transporter 4 (OAT4), organic anion transporting polypeptide 2B1 (OATP2B1) and monocarboxylate transporter 1 (MCT1). The arrows show the direction of substrate transport through the cells.

User rates of drugs associated with placental transporters among cases and the referent population

Characteristics of children born to case mothers and to mothers in the referent population are shown in **Table 1**. The mothers in the case group were slightly older than the mothers in the referent population (30.2 ± 4.6 years and 29.5 ± 4.9 years, respectively, $p < 0.01$). The majority of cases were liveborn while all the children in the referent population were live born. The most common types of anomalies among the case group were heart and limb anomalies (around 25% each), followed by anomalies in the digestive system (12.3%).

Out of 147 drugs associated with placental transporters, 58 were used by at least one mother in either the cases or the referent population, as listed in **Table 2**. Only 28 of the drugs were used by at least one mother in both groups. Table 3 shows the percentage of mothers who used at least one of the drugs, according to their substrate specificity to each placental transporter protein. We found no users of drugs that are substrates/inducers or inducers of these transporters. The use of MRP1 drug substrates was most common as compared to substrates of other transporters; it was observed in 6% of the case mothers and 8% of the referent population. The highest user rate was for folic acid (prescribed), as one of the substrates of MRP1. BCRP substrates were used in 3% and 3.6% of the case and referent population, respectively. The percentage resulted largely from the use of nitrofurantoin (2.3% and 2.9% in cases and referent population, respectively). Restricting the analysis for liveborn cases only showed a slight reduction in user rates for each transporter (**Table 2 and 3**).

Placental transporter-mediated drug interactions and the risk of congenital anomalies

Drug interaction analysis can only be done for MRP1, BCRP and MCT1 substrates. There were no users of OCT3, OAT4, OATP2B1 and MCT4 substrates in combination with an inhibitor to calculate the OR. Due to limited sample size, we were not able to show any significant increase in the risk of congenital anomalies with the use of drugs transported by each transporter in combination with the inhibitors.

Table 1: Characteristics of children born to case mothers and referent population mothers

Characteristics	Cases (N=5,131)	Referent population (N=31,055)
Maternal age at delivery*, mean years ± s.d	30.2 ± 4.6	29.5 ± 4.9
Gender, N (%)		
Boy	2,864 (55.8)	16,064 (51.7)
Girl	2,259 (44.0)	14,991 (48.3)
Missing data	8 (0.2)	-
Year of birth, N (%)		
1997-1998	665 (13.0)	5,736 (18.5)
1999-2000	695 (13.5)	5,492 (17.7)
2001-2002	634 (12.4)	4,551 (14.7)
2003-2004	620 (12.1)	3,954 (12.7)
2005-2006	637 (12.4)	3,247 (10.5)
2007-2008	576 (11.2)	2,927 (9.4)
2009-2010	658 (12.8)	2,805 (9.0)
2011-2012	527 (10.3)	1,983 (6.4)
2013	119 (2.3)	360 (1.2)
Type of birth, N (%)		
Live birth	4,805 (93.6)	31,055 (100.0)
Termination of pregnancy	224 (4.4)	0
Stillbirth	65 (1.3)	0
Miscarriage (>24 weeks)	37 (0.7)	0
Types of anomalies[a], N (%)		
Heart	1,377 (26.8)	-
Limb	1,228 (23.9)	-
Digestive	633 (12.3)	-
Urinary	563 (11.0)	-
Clefts	457 (8.9)	-
Genital	444 (8.7)	-
Central nervous system	383 (7.5)	-
Eye, ear, face and neck	175 (3.4)	-
Respiratory	91 (1.8)	-

*p-value < 0.01; s.d, standard deviation; [a] cases with multiple congenital anomalies are represented in more than one anomaly group.

Table 2: User rates of selected drugs among cases and referent population, according to substrate specificity to each placental transporter

No	Drugs	Transporter proteins										Number of users, n (%)		
		BCRP	OCT3	OAT4	MRP1	OATP2B1	ENT1	MCT1	MCT4	MCT8	MCT10	All cases (N=5,131)	Liveborn (N=4,805)	Referent (N=31,055)
1	Acetazolamide			Inhibitor								0	0	1 (0.003)
2	Albendazole	Substrate										0	0	1 (0.003)
3	Amantadine		Inhibitor									0	0	1 (0.003)
4	Amitriptyline		Substrate/ Inhibitor									10 (0.2)	10 (0.2)	97 (0.3)
5	Atorvastatin					Substrate/ Inhibitor		Substrate	Inhibitor			2 (0.04)	2 (0.04)	12 (0.04)
6	Bumetanide			Inhibitor								1 (0.02)	1 (0.02)	1 (0.003)
7	Candesartan			Inhibitor								0	0	5 (0.02)
8	Captopril			Inhibitor								0	0	1 (0.003)
9	Ceftriaxone			Inhibitor								1 (0.02)	1 (0.02)	2 (0.006)
10	Cimetidine	Substrate	Substrate/ Inhibitor									2(0.04)	2(0.04)	20 (0.1)
11	Ciprofloxacin	Substrate										2 (0.04)	2 (0.04)	41 (0.1)
12	Citalopram		Substrate/ Inhibitor									17 (0.3)	14 (0.3)	102 (0.3)
13	Clonidine		Substrate/ Inhibitor									0	0	3 (0.01)
14	Cyclosporine	Inhibitor			Inhibitor	Inhibitor						1 (0.02)	1 (0.02)	4 (0.01)
15	Diltiazem		Substrate/ Inhibitor									1 (0.02)	1 (0.02)	2 (0.006)
16	Dipyridamole	Inhibitor					Inhibitor	Substrate				0	0	5 (0.02)
17	Efavirenz	Substrate/ Inhibitor			Substrate/ Inhibitor							0	0	1 (0.003)
18	Ephinephrine		Substrate									0	0	10 (0.03)
19	Erythromycin	Substrate										14 (0.3)	14 (0.3)	77 (0.2)
20	Estrone					Substrate/ Inhibitor						0	0	1 (0.003)

No	Drugs	Transporter proteins										Number of cases, n (%)		
		BCRP	OCT3	OAT4	MRP1	OATP2B1	ENT1	MCT1	MCT4	MCT8	MCT10	All cases (N=5,131)	Liveborn (N=4,805)	Referent (N=31,055)
21	Famotidine		Inhibitor									0	0	5 (0.02)
22	Fexofenadine		Substrate/ Inhibitor			Substrate/ Inhibitor						7 (0.1)	7 (0.1)	80 (0.3)
23	Flecainide		Substrate/ Inhibitor									0	0	1 (0.003)
24	Fluvastatin					Substrate			Inhibitor			0	0	1 (0.003)
25	Folic acid				Substrate							307 (6)*	288 (6)*	2471 (8)
26	Furosemide	Substrate		Inhibitor								1 (0.02)	0	14 (0.04)
27	Gabapentin							Inhibitor				0	0	4 (0.01)
28	Gemfibrozil					Inhibitor						0	0	1 (0.003)
29	Glibenclamide	Substrate				Substrate/ Inhibitor						0	0	3 (0.01)
30	Hydrochloro-thiazide	Substrate										5 (0.1)	4 (0.1)	35 (0.1)
31	Ibuprofen							Inhibitor				63 (1.2) *	59 (1.2)	850 (2.7)
32	Imipramine		Substrate/ Inhibitor									0	0	4 (0.01)
33	Indomethacin				Inhibitor							7 (0.1)	7 (0.1)	18 (0.1)
34	Ketoprofen			Substrate/ Inhibitor				Inhibitor		Inhibitor	Inhibitor	0	0	3 (0.01)
35	Lamivudine	Substrate	Substrate/ Inhibitor		Substrate/ Inhibitor							0	0	1 (0.003)
36	Lopinavir	Substrate/ Inhibitor			Substrate/ Inhibitor	Substrate/ Inhibitor						0	0	1 (0.003)
37	Losartan			Inhibitor								0	0	4 (0.01)
38	Metformin		Substrate/ Inhibitor									12 (0.2)	12 (0.2)	33 (0.1)
39	Methotrexate	Substrate		Substrate/ Inhibitor	Substrate							0	0	1 (0.003)

No	Drugs	Transporter proteins										Number of cases, n (%)		
		BCRP	OCT3	OAT4	MRP1	OATP2B1	ENT1	MCT1	MCT4	MCT8	MCT10	All cases (N=5,131)	Liveborn (N=4,805)	Referent (N=31,055)
40	Nelfinavir	Inhibitor	Substrate/ Inhibitor			Substrate/ Inhibitor						0	0	1 (0.003)
41	Nitrofurantoin	Substrate										119 (2.3)	107 (2.2)	908 (2.9)
42	Norfloxacin	Substrate										4 (0.1)	4 (0.1)	36 (0.1)
43	Ofloxacin	Substrate										1 (0.02)		13 (0.04)
44	Omeprazole	Inhibitor										48 (0.9)	43 (0.9)	290 (0.9)
45	Pravastatin			Substrate/ Inhibitor		Substrate/ Inhibitor		Substrate				0	0	1 (0.003)
46	Progesterone		Inhibitor									72 (1.4)	67 (1.4)	295 (0.9)
47	Ranitidine		Substrate/ Inhibitor									7 (0.14) *	7 (0.1)	140 (0.5)
48	Rifampicin		Inhibitor			Inhibitor						0	0	2 (0.007)
49	Rosuvastatin	Substrate				Substrate						0	0	3 (0.01)
50	Salicylic acid (aspirin)							Substrate				3 (0.17)*	2 (0.04)	1 (0.003)
51	Simvastatin					Inhibitor			Inhibitor			3 (0.06)	3 (0.1)	19 (0.1)
52	Sulfasalazine	Substrate										8 (0.2)	7 (0.1)	25 (0.1)
53	Tacrolimus	Inhibitor										0	0	3 (0.01)
54	Tenofovir		Substrate/ Inhibitor		Substrate/ Inhibitor							0	0	1 (0.003)
55	Tetracycline			Substrate/ Inhibitor								4 (0.1)	4 (0.1)	27 (0.1)
56	Valproic acid			Substrate/ Inhibitor				Substrate	Substrate			20 (0.4)	17 (0.4)	29 (0.1)
57	Valsartan			Inhibitor								0	0	2 (0.006)
58	Verapamil		Substrate/ Inhibitor									0	0	9 (0.03)

BCRP, breast cancer resistance protein; OCT, organic cation transporter; OAT, organic anion transporter; MRP, multidrug resistance-associated protein; OATP, organic anion transporting polypeptide; ENT, equilibrative nucleoside transporter; MCT, monocarboxylate transporter; * prescribed.

Table 3: User rates of drugs associated with placental transporter in the first trimester of pregnancy among cases and referent population

Placental Transporters	Substrate#, n (%)			Substrate/Inhibitor#, n (%)			Inhibitor#, n (%)		
	All cases (N=5,131)	Liveborn cases (N=4,805)	Referent population (N=31,055)	All cases (N=5,131)	Liveborn cases (N=4,805)	Referent population (N=31,055)	All cases (N=5,131)	Liveborn cases (N=4,805)	Referent population (N=31,055)
BCRP	153 (3.0)	137 (2.9)	1,131 (3.6)	0	0	2 (0.01)	49 (1.0)	44(0.92)	302 (1.0)
OCT3	0	0	10 (0.03)	56 (1.1)	53 (1.1)	480 (1.5)	72 (1.4)	67 (1.4)	303 (1.0)
OAT4	0	0	0	24 (0.47)	21 (0.44)	60 (0.19)	3 (0.06)	2 (0.04)	28 (0.09)
MRP1	307 (6.0)	288 (6.0)	2471 (8.0)	0	0	2 (0.01)	8 (0.16)	8 (0.17)	21 (0.07)
OATP2B1	0	0	3 (0.01)	9 (0.18)	9 (0.19)	100 (0.32)	4 (0.08)	4 (0.08)	25 (0.08)
ENT1	0	0	0	0	0	0	0	0	5 (0.02)
MCT1	25 (0.55)	21 (0.44)	48 (0.15)	0	0	0	63 (1.2)	59 (1.2)	857 (2.8)
MCT4	20 (0.39)	17 (0.35)	29 (0.09)	0	0	0	5 (0.10)	5 (0.10)	32 (0.10)
MCT8 & MCT10*	0	0	0	0	0	0	0	0	3 (0.01)

BCRP, breast cancer resistance protein; OCT, organic cation transporter; OAT, organic anion transporter; MRP, multidrug resistance-associated protein; OATP, organic anion transporting polypeptide; ENT, equilibrative nucleoside transporter; MCT, monocarboxylate transporter; # same mother who used more than one drug that belongs to the same substrate classification was counted once; * same drug substrates for both transporters.

DISCUSSION

Placental transporters may potentially play a role in fetal drug transfer in view of the vast range of drugs transported by these transporters. For ten placental transporters included in this study, 28 drugs were reported in *in-vitro* or *ex-vivo* studies to be substrates or inhibitors, and these were used by at least one pregnant woman in both the case group and the referent population. For each placental transporter, the number of mothers who used drug substrates were generally lower than for P-gp, (the use of P-gp drug substrates was 10% in cases and 12% in the referent population) (11). This is probably because the substrates for those transporters are much less studied and therefore much less is known about them as compared to P-gp substrates.

Knowledge about the expression of most of the placental transporters in this study is relatively new, and their contribution to drug transport in the placenta has not been completely characterized. Despite that, transporter-mediated drug interactions can potentially cause clinically relevant changes in drug pharmacokinetics and drug exposure, as previously observed for P-gp, BCRP and MRPs (5,30). In this study, we did not find significant changes in the risk for congenital anomalies by inhibitors of the placental transporters studied, possibly due to the limited number of users in each drug interaction pattern.

There are also several other factors that should be taken into consideration in interpreting the role of placental transporters in the fetal drug exposure. First, the same efflux and influx transporters are also present in other tissues (i.e. intestine and liver), which are important for the distribution and elimination of their substrates. Drug interactions in these tissues, apart from the placenta, may affect substrate exposure to the fetus. However, these interactions did not warrant pharmacovigilance measures so far, therefore we do not expect these interactions to have a significant impact on our results. Second, there is high inter-individual variation in the expression of these transporters due to genetic variability in the genes encoding for them (4,12). Genotype-dependent transporter-mediated drug interactions have not yet been well studied, but the effect was already observed for fexofenadine and OATP2B1 inhibition (31), and for metformin and OCT2 inhibition (32). Third, drug pharmacokinetics in the maternal circulation are also altered during pregnancy due to normal physiological changes (33,34), and possible drug-drug interaction involving metabolic enzymes, i.e. CYP450. However, using the software-supported medications surveillance system in the Netherlands, these interactions may have been avoided during the prescribing and dispensing process. Therefore, we assumed that, at least from 2002, the clinically significant CYP-mediated drug interactions had already been avoided (35). Fourth, placental transporters often have broad and overlapping

substrate specificities, and the net effect of drug interactions involving substrates with more than one transporter is difficult to measure.

Our study has several strengths and limitations. One of the strengths is that the information on drug use was well documented in both databases. Duration of drug use is well documented with minimal recall bias, and the inclusion of mother-children pair was done over the same period of years. Further, our study is among the first to investigate the role of transporter proteins in the placenta using a population-based study. Our knowledge of fetal transfer of drugs was previously based on *in vitro* studies using cell lines and *ex vivo* placental models, which do not take into consideration the other clinical parameters involved (36,37). Moreover, limited methods can be used to study the role of transporters in the early stage of pregnancy, which is a crucial period for fetal development.

One limitation of our study is the potential for misclassification of drug exposure in the prescription database (Pregnancy IADB.nl) because we cannot be sure that the mothers actually took the drugs dispensed. Further, the period of exposure was an estimation calculated from the date of delivery with the assumption of 273 days of gestation. If the mothers were classified as exposed when they were not, it would lead to an underestimation of the observed risk. Another limitation is that placental transporter expression, instead of protein level, is used and the assumption of drug interactions at the placenta is based on drugs prescribed and not on measured drug concentrations. Confounding by indication is also impossible to address because nothing is known in our databases about the medical condition for which the drug was prescribed and used. Finally, as acknowledged earlier, despite a large number of cases and referents, due to a wide variety of exposures, we had limited power to detect statistically significant effects.

In conclusion, we classified drugs based on their substrate specificity to several placental transporter proteins, and described the use of these drugs during the first trimester of pregnancy. Using the same approach as in our previous study on P-gp, we were unable to find a significant association between the inhibition of these transporters and the risk of congenital anomalies. We did not have enough power to draw conclusions on the causality, and larger databases are needed to answer this question. However, the list of substrates for each transporter may not be complete, and therefore the effect of drug interactions may be underestimated. Nonetheless, knowledge about transporter-mediated drug interactions in the placenta is clearly important for drugs with known risk of teratogenicity. Larger-scale databases are needed in the future to further denote the role of these transporters in fetal drug transport.

REFERENCES

1. Staud F, Cerveny L, Ceckova M. Pharmacotherapy in pregnancy; effect of ABC and SLC transporters on drug transport across the placenta and fetal drug exposure. J Drug Target. 2012 Nov;20(9):736–63.

2. Iqbal M, Audette MC, Petropoulos S, Gibb W, Matthews SG. Placental drug transporters and their role in fetal protection. Placenta. Elsevier Ltd; 2012 Mar;33(3):137–42.

3. Vahakangas K, Myllynen P. Drug transporters in the human blood-placental barrier. Br J Pharmacol. 2009;158(3):665–78.

4. Myllynen P, Immonen E, Kummu M, Vähäkangas K. Developmental expression of drug metabolizing enzymes and transporter proteins in human placenta and fetal tissues. Expert Opin Drug Metab Toxicol. 2009 Dec;5(12):1483–99.

5. Endres CJ, Hsiao P, Chung FS, Unadkat JD. The role of transporters in drug interactions. Eur J Pharm Sci. 2006 Apr;27(5):501–17.

6. Nanovskaya T, Nekhayeva I, Karunaratne N, Audus K, Hankins GD V, Ahmed MS. Role of P-glycoprotein in transplacental transfer of methadone. Biochem Pharmacol. 2005 Jun 15;69(12):1869–78.

7. May K, Minarikova V, Linnemann K, Zygmunt M, Kroemer HK, Fusch C, et al. Role of the multidrug transporter proteins ABCB1 and ABCC2 in the diaplacental transport of talinolol in the term human placenta. Drug Metab Dispos. 2008 Apr;36(4):740–4.

8. Ceccaldi P-F, Gavard L, Mandelbrot L, Rey E, Farinotti R, Treluyer J-M, et al. Functional role of p-glycoprotein and binding protein effect on the placental transfer of lopinavir/ritonavir in the ex vivo human perfusion model. Obstet Gynecol Int. 2009 Jan;doi 10.115:1–6.

9. Mölsä M, Heikkinen T, Hakkola J, Hakala K, Wallerman O, Wadelius M, et al. Functional role of P-glycoprotein in the human blood-placental barrier. Clin Pharmacol Ther. 2005 Aug;78(2):123–31.

10. Holcberg G, Sapir O, Tsadkin M, Huleihel M, Lazer S, Katz M, et al. Lack of interaction of digoxin and P-glycoprotein inhibitors, quinidine and verapamil in human placenta in vitro. Eur J Obstet Gynecol Reprod Biol. 2003 Aug;109(2):133–7.

11. Daud ANA, Bergman JEH, Bakker MK, Wang H, Kerstjens-Frederikse WS, de Walle HEK, et al. P-Glycoprotein-Mediated Drug Interactions in Pregnancy and Changes in the Risk of Congenital Anomalies: A Case-Reference Study. Drug Saf. 2015;38(7):651–9.

12. Muller F, Fromm MF. Transporter-mediated drug–drug interactions. Pharmacogenomics. 2011;12(7):1017–37.

13. König J, Müller F, Fromm MF. Transporters and drug-drug interactions: important determinants of drug disposition and effects. Pharmacol Rev. 2013;65(3):944–66.

14. European Surveillance of Congenital Anomalies (EUROCAT). Subgroups of Congenital Anomalies (Version 2012). Available from: http://www.eurocat-network.eu/content/EUROCAT-Guide-1.4-Section-3.3.pdf. Last accessed 10 June 2016.

15. Visser ST, Schuiling-Veninga CC, Bos JH, de Jong-van den Berg LTW, Postma MJ. The population-based prescription database IADB.nl : its development, usefulness in outcomes research and challenges. Expert Rev Pharmacoeconomics Outcome Res. 2013;13(3):285–92.

16. Schirm E, Tobi H, de Jong-van den Berg LTW. Identifying parents in pharmacy data: a tool for the continuous monitoring of drug exposure to unborn children. J Clin Epidemiol. 2004 Jul;57(7):737–41.

17. Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. Drug Metab Pharmacokinet. 2005 Dec;20(6):452–77.

18. Nishimura M, Naito S. Tissue-specific mRNA expression profiles of human solute carrier transporter superfamilies. Drug Metab Pharmacokinet. 2008;23(1):22–44.

19. Nishimura M, Suzuki S, Satoh T, Naito S. Tissue-Specific mRNA Expression Profiles of Human Solute Carrier 35 Transporters. Drug Metab Pharmacokinet. 2009;24(1):91–9.

20. Yeboah D, Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, et al. Expression of breast cancer resistance protein (BCRP / ABCG2) in human placenta throughout gestation and at term before and after labor. Can J Physiol Pharmacol. 2006;84:1251–8.

21. Lee N, Hebert MF, Prasad B, Easterling TR, Kelly EJ, Unadkat JD, et al. Effect of gestational age on mRNA and protein expression of polyspecific organic cation transporters during pregnancy. Drug Metab Dispos. 2013;41(12):2225–32.

22. Ugele B, Bahn A, Rex-Haffner M. Functional differences in steroid sulfate uptake of organic anion transporter 4 (OAT4) and organic anion transporting polypeptide 2B1 (OATP2B1) in human placenta. J Steroid Biochem Mol Biol. 2008;111:1–6.

23. Hahnova-Cygalova L, Ceckova M, Staud F. Fetoprotective activity of breast cancer resistance protein (BCRP, ABCG2): expression and function throughout pregnancy. Drug Metab Rev. 2011 Feb;43(1):53–68.

24. Staud F, Ceckova M. Regulation of drug transporter expression and function in the placenta. Expert Opin Drug Metab Toxicol. 2015;11(4):533–55.

25. Vähäkangas K, Myllynen P. Drug transporters in the human blood-placental barrier. Br J Pharmacol. 2009 Oct;158(3):665–78.

26. Mao Q, Unadkat JD. Role of the Breast Cancer Resistance Protein (ABCG2) in Drug Transport-An Update. AAPS J. 2015;17(1):65–82.

27. Ho RH, Kim RB. Transporters and drug therapy: Implications for drug disposition and disease. Clin Pharmacol Ther. 2005;78(3):260–77.

28. Roth M, Obaidat A, Hagenbuch B. OATPs, OATs and OCTs: The organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. Br J Pharmacol. 2012;165(5):1260–87.

29. Koepsell H, Endou H. The SLC22 drug transporter family. Pflugers Arch. 2004 Feb;447(5):666–76.

30. Rubinchik-Stern M, Eyal S. Drug Interactions at the Human Placenta: What is the Evidence? Front Pharmacol. 2012;3:1–7.

31. Imanaga J, Kotegawa T, Imai H, Tsutsumi K, Yoshizato T, Ohyama T, et al. The effects of the SLCO2B1 c.1457C > T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans. Pharmacogenet Genomics. 2011 Feb;21(2):84–93.

32. Wang Z-J, Yin OQP, Tomlinson B, Chow MSS. OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine. Pharmacogenet Genomics. 2008;18(7):637–45.

33. Unadkat JD, Dahlin A, Vijay S. Placental drug transporters. Curr Drug Metab. 2004 Feb;5(1):125–31.

34. Xia B, Heimbach T, Gollen R, Nanavati C, He H. A simplified PBPK modeling approach for prediction of pharmacokinetics of four primarily renally excreted and CYP3A metabolized compounds during pregnancy. AAPS J. 2013;15(4):1012–24.

35. Van Roon EN, Flikweert S, Le Comte M, Langendijk PNJ, Kwee-Zuiderwijk WJM, Smits P, et al. Clinical relevance of drug-drug interactions: A structured assessment procedure. Drug Saf. 2005;28(12):1131–9.

36. Prouillac C, Lecoeur S. The Role of the Placenta in Fetal Exposure to Xenobiotics : Importance of Membrane Transporters and Human Models for Transfer Studies. Drug Metab Dispos. 2010;38(10):1623–35.

37. Isoherranen N, Thummel KE. Drug metabolism and transport during pregnancy : How does drug disposition change during pregnancy and what are the mechanisms that cause such changes ? Drug Metab Dispos. 2013;41:256–62.

chapter 4

Pharmacogenetics of drug-induced birth defects:

The role of polymorphisms of placental transporter proteins

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ABSTRACT

One of the ongoing issues in perinatal medicine is the risk of birth defects associated with maternal drug use. The teratogenic effect of a drug depends, apart from other factors, on the exposition of the fetus to the drug. Transporter proteins are known to be involved in the pharmacokinetics of drugs and have an effect on drug level and fetal drug exposure. This condition may subsequently alter the risk of teratogenicity, which occurs in a dose-dependent manner. This review focuses on the clinically important polymorphisms of transporter proteins and their effects on the mRNA and protein expression in placental tissue. We also propose a novel approach on how the different genotypes of the polymorphism can be translated into phenotypes to facilitate genetic association studies. The last section looks into the recent studies exploring the association between P-glycoprotein polymorphisms and the risk of fetal birth defects associated with medication use during pregnancy.

INTRODUCTION

Transporter proteins are expressed in many different tissues, such as the liver, lungs, pancreas, kidneys, intestines and brain. These transporters are also expressed in the placenta, in which they are involved in the transport of endogenous compounds and drugs across the placenta. Owing to the important role of the transporters in maternal-to-fetal drug disposition, they have been well-characterized with respect to structure, localization and physiological activity in the placenta (1–5). These transporters are recognized as protective barriers to the fetus from the harmful effects of the drugs taken by the mother. The exposure to teratogenic drugs in a specific dose range may interfere with the development of fetal organ systems, leading to structural anomalies. The fetus is most susceptible to the teratogenesis during the crucial organogenesis stage, which occurs from implantation to 54–60 days after conception (6). In this early stage of pregnancy, some of the transporter proteins are already expressed in the placental tissue. In addition, the expression pattern throughout pregnancy varies between transporters, which will be discussed in detail for each transporter.

It is increasingly recognized that the activity of these transporters is affected by genetic polymorphisms. Studies on the pharmacogenetics of these transporters are gaining momentum, particularly in their importance in transporter expression and function, as well as the pharmacotherapy of substrate drugs (7–11). The knowledge so far has promoted a notion on the effect of polymorphisms on the drug disposition in the placenta and the fetal exposure to teratogenic drugs. Some studies have pursued the significance of efflux transporter protein polymorphisms in placental drug transport. The changes in the expression or function of the transporter proteins are known to alter the amount of drugs transferred into the placenta *in vitro* or *ex vivo* (12). However, it is relatively difficult to study the effects of these polymorphisms on the protein function in a clinical setting because the clinical outcome may also involve other mechanisms including the pharmacokinetics and pharmacodynamics of drug substrates.

The objective of this review is to gain insight into the current knowledge of the functional polymorphisms of transporter proteins in the placenta and potential applications in the risk determination of drug-induced birth defects. We also propose a novel approach to group polymorphisms according to functionality, herein referred to as 'phenotype groups', instead of studying the polymorphisms separately to facilitate studies on the association between transporter polymorphisms and their functional effects.

METHODS

A literature review was performed from February until September 2013 using the PubMed database. Articles are searched using the combinations of the following keywords: 'p-glycoprotein [MeSH]', 'ABCG2', 'MRP*', 'ABCC*', 'organic anion transporters [MeSH]', 'organic cation transport proteins [MeSH]', 'human multidrug and toxin extrusion protein 1', 'genetic polymorphism [MeSH]', 'ethnic groups', 'placenta' and 'transport'. See **Figure 1** for the search strategy outline.

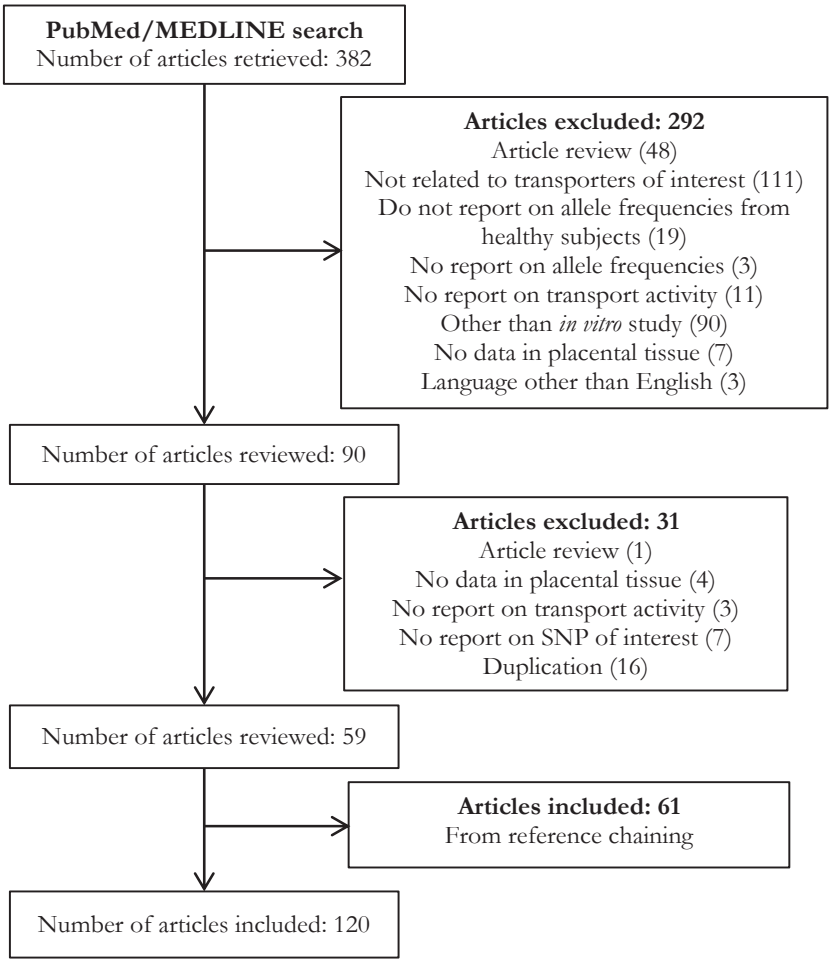

Figure 1: Search strategy

Pharmacogenetics of transporter proteins

Placental transporter proteins & known polymorphisms

Various transporter proteins are expressed and localized in the placental syncytiotrophoblast, a multinucleated cell formed by the fusion of the differentiating cytotrophoblast. The plasma membrane of the syncytiotrophoblast consists of two distinct regions, the brush border apical membrane, which is facing the maternal blood circulation, and the basal/basolateral membrane, which is facing the fetal blood circulation. To reach the fetus, any compound from the maternal circulation has to pass through the brush border apical membrane and subsequently the basal membrane of the placenta, both endowed with the transporters mentioned (1,3).

Relevant for drug transport, syncytiotrophoblasts express two major types of transporter proteins: ATP- binding cassette (ABC) transporter proteins and solute carrier (SLC) transporter proteins **(Figure 2)**. These transporters are specifically localized at one of the membrane regions to facilitate the transport of various compounds, including nutrients and endogenous substrates such as carbohydrates and proteins, from the maternal to the fetal blood circulation or vice versa. Another important role of the transporters is to provide a protection barrier for the fetus against harmful effects of drugs taken by the mother.

Additional to passive diffusion through the cell membrane, the transfer of drugs across the placenta is also facilitated by the transporter proteins, either with active transport via the ABC transporters or the facilitated transport via the SLC transporters. It is important to outweigh the risks against the benefits when prescribing certain drugs such as antidepressants, antiepileptics and immunosuppressants to pregnant women, because these drugs have been associated with an increased risk for birth defects. The majority of these drugs is lipid soluble, and will normally diffuse into the placental syncytiotrophoblast, and this is where the role of transporters takes place. Drugs that are substrates for the efflux transporter will be transported back into the maternal circulation, while drugs that have diffused into the fetal circulation will be transported back into the syncytiotrophoblast. This mechanism reduces the amount of drug entering the fetal circulation and hence the fetal exposure to any harmful effects associated with the drugs.

As the placenta is mainly of fetal origin, we can expect that the polymorphism in the fetal gene is responsible for the expression and activity of placental transporters. A polymorphism may cause a substitution of amino acid in the formation of protein, which can be seen with nonsynonymous SNPs, and may subsequently have an effect on the expression or function of the protein.

Meanwhile, the synonymous SNPs that cause no changes in amino acid sequence have also been studied for their effect on gene and protein expression.

Any change in the expression or function of these transporters may influence the entry of drugs into the fetal circulation, and thereby have an effect on the fetal exposure to the drugs, which may consequently alter the risk of birth defects associated with teratogenic drugs. Several polymorphisms have been identified and a number of them have been shown to be functionally important in the transport of endogenous substrates and drugs (**Appendix 3.1**).

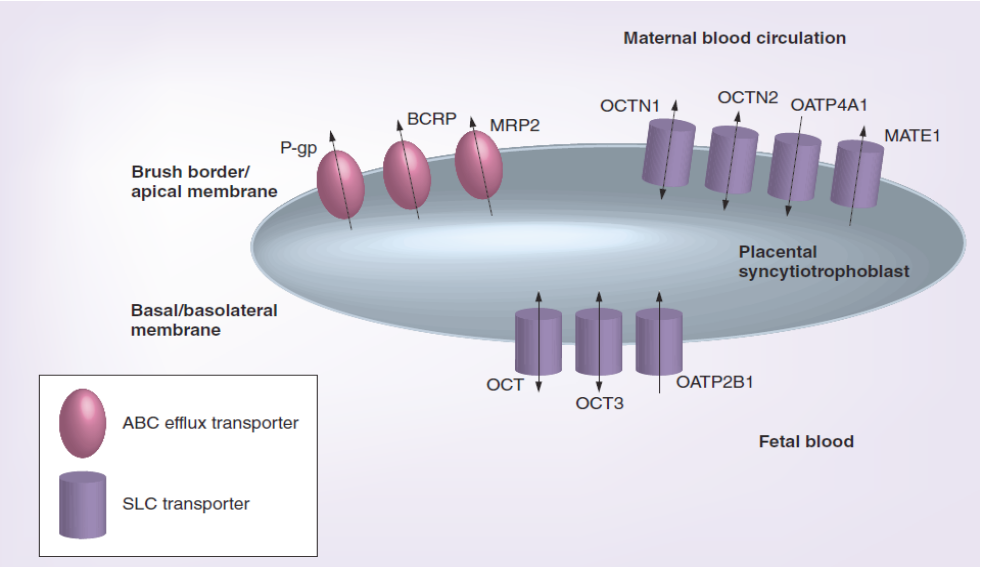

Figure 2: Localization of ABC efflux transporters and SLC transporters in the human placenta. ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; MATE, Multidrug and toxin extrusion transporter; MRP, Multidrug resistance-associated protein; OATP, Organic anion transporting polypeptide; OCT, Organic cation transporter; OCTN, Organic cation/carnitine transporter; P-gp, P-glycoprotein; SLC, solute carrier

P-glycoprotein (P-gp)

P-gp, encoded by the *ABCB1* gene, belongs to the ATP-binding cassette transporters and is localized at the maternal-facing apical membrane of the syncytiotrophoblast (13). P-gp acts as an efflux transporter and is involved in the transport of a wide variety of compounds with different chemical structures, including a large number of therapeutic drugs (5). The expression is higher in preterm pregnancy and decreases with gestational age, suggesting that it plays a role in protecting the fetus from teratogenic drugs during the crucial organogenesis period (14).

The first evidence of *ABCB1* gene polymorphism was reported by Mickley and colleagues, when they found two SNPs, 2677G>T in exon 21 and 2995G>A in exon 24 (15). Subsequently numerous findings on other SNPs were reported. The synonymous 3435C>T in exon 26 is the most studied SNP of P-gp, initiated by the findings from Hoffmeyer and colleagues who reported the association of the 3435T variant allele with lower expression of P-gp in duodenal tissue (16).

Effect of polymorphisms in the human placenta: mRNA/protein expression

Several studies have investigated the effect of 3435C>T in human placental tissue on mRNA and protein expression, but similar to other tissues, the results are conflicting (**Appendix 3.2**). The variant allele has been associated with lower mRNA expression in the full-term placenta trophoblast tissue compared with the wild-type allele (17), but this association has not been confirmed in another study (18). The same allele has also been associated with both increased and decreased P-gp protein expression in the placenta, although the studies used different types of placental tissue (18–20).

The expression of P-gp in the placenta does not depend only on the fetal genotype. Investigating both maternal and fetal genotypes, Hitzl et al. have found lower placental P-gp expression when both mother and infant were homozygous or heterozygous carriers of the variant allele of 3435C>T. These results suggest that it is necessary to take the maternal genotype into account when determining the effect of polymorphism in placental tissue, with a probable role of maternal blood vessels in expressing P-gp, apart from the syncytiotrophoblast (18). The study also looked into the effect of haplotypes, a set of SNPs on a single chromosome or chromosome pair. In the 2677G>T/3435C>T haplotype, the maternal variant haplotype has been shown to be associated with lower mRNA and protein expression. However, the association was neither present in the fetal haplotype nor the combination of both maternal and fetal genotypes (18).

A significant effect on P-gp expression was also observed with 1236C>T, in which the T allele was associated with significantly lower P-gp expression compared with the wild-type C allele (20). A SNP at the promoter region, the -129T>C, has been found to cause lower gene transcription efficiency that resulted in a twofold decrease in protein expression. The promoter region plays a crucial part as an initiator in the protein transcription process (21).

Even though mRNA is important for protein translation and expression, there was no correlation found between P-gp mRNA and protein expression level (18). Since most of the studies quantified mRNA expression in full-term placenta samples, the fact that mRNA undergoes rapid degradation after delivery compared with protein may also cause the actual level of mRNA to be prone to bias (12).

Effect of polymorphisms in human placenta: substrate transport

In the human placenta, and similarly in transfected cell lines, effects of P-gp polymorphisms on efflux activity seem to be independent of their effect on mRNA and protein expression (19–20,22). Thus, the level of protein expression is not a good predictor for its efflux activity of drug substrate through the placenta (**Appendix 3.3**). The 3435C>T polymorphism has been reported to cause an increase in paclitaxel efflux activity (20), but not for saquinavir (19). However, an increase in transplacental transfer was observed for quetiapine, meaning that the efflux activity, on the contrary, was somehow decreased (23). Although both the studies on saquinavir and quetiapine used a reproducible and well-established placental perfusion method, the sample sizes were small and other transporters, which were not studied, could also play a role in the efflux of these substrate drugs (19,23). Hemauer and colleagues, who used paclitaxel as a well-established P-gp substrate with bigger samples of placental brush border membrane vesicles, found an increased function of this transporter associated with 3435C>T, 1236C>T and 2677G>T/A polymorphisms. The function was measured by the transport of paclitaxel into the inside-out vesicles (20).

There are various limitations in studies using placental tissues in genotyping and protein quantification analysis (as reviewed by Hutson et al. 2010) (12). A major concern is the small number of placental tissue samples. As one example, the expression of P-gp decreases with gestational age, therefore age-matched samples are required to obtain a significant association. The second concern is the environmental factors that can also affect the regulation and transport activity, especially with the use of medications known to be inducers or inhibitors of transporter proteins. Another concern is that the results are susceptible to the substrate-dependent effect. A different effect on transport activity has been observed for different substrates,

probably results from the transport activity of secondary transporters for which the drug is also a substrate (8). An alteration in P-gp efflux activity for a substrate with high affinity to it can be assumed to be more significant compared with other substrates with lower affinity, in which the efflux transport by other transporters may have countered the previous alteration. Therefore proper selection of the substrate is important in assessing the changes in P-gp activity associated with polymorphism.

Breast cancer resistance protein (BCRP)

BCRP is a 75 kDa membrane protein located at the apical surface of the syncytiotrophoblast (24,25) and the luminal membrane of fetal endothelial cells in terminal and intermediate villi (26). The expression pattern of placenta BCRP during pregnancy is not conclusive, with two studies reporting the constant levels of mRNA throughout pregnancy (27,28) while the latter study also reported an increase in BCRP expression with advancing gestational age. BCRP shows considerable overlap in substrate transport specificity with P-gp, including many anticancer, antiviral and antibiotic drugs (29). BCRP is encoded by the *ABCG2* gene, and several polymorphisms of this gene were found more often in the Asian population, for example 421C>A, 34G>A and 376C>T (30,31).

Effect of polymorphisms in the human placenta: mRNA/protein expression

Only one study has included the effect of *ABCG2* polymorphisms on the mRNA and protein expression of BCRP in the human placenta. As reported by Kobayashi et al., 421C>A was significantly associated with changes in the BCRP protein level; the homozygous C allele expressed the highest level, followed by heterozygous, while the A allele expressed the lowest. Since no alteration in mRNA expression has been associated with this SNP, it was postulated to be the effect of post-transcriptional regulation. The 376 C>T polymorphism was detected in only two individuals in this study; hence a statistical analysis cannot be carried out. Nevertheless, both who were present with the heterozygous genotype exhibit lower protein levels (31).

Multidrug resistance-associated proteins (MRPs)

The MRPs are expressed by *ABCC* family genes and consist of nine membrane proteins important in the efflux transport of anion conjugates (5). At present, human placenta at full-term is known to express at least four members of the MRP protein family: MRP1, MRP2, MRP3 and MRP5. Because MRP2 is the only MRP with known functional polymorphisms studied in human placenta, we will focus only on this transporter.

MRP2 (*ABCC2*) is located at the apical membrane of the syncytiotrophoblast facing the maternal blood (32,33) and in contrast to P-gp, its expression increases during gestation, suggesting that it is important in fetal protection during late pregnancy (33). SNP analysis carried out by Ito and colleagues has listed six nonsynonymous SNPs in 48 healthy Japanese, and a year later Itoda et al. have reported 23 SNPs in the exonic region and four SNPs in the promoter region (34,35). A notable difference of allele frequency between different ethnicities applies to 3972C>T, which was present in approximately 34–36% of Caucasians, in approximately 22% of Japanese and in less than 3% of southeastern populations (34–36). Meanwhile, several SNPs were only found in Asians, while Caucasians and other ethnicities were mainly endowed with wild-type alleles (34–37).

Effect of polymorphisms in the human placenta: mRNA/protein expression

The effect of the three *ABCC2* SNPs, -24C>T, 1249G>A and 3972C>T on mRNA and MRP2 expression in human placenta is not remarkable, except for the influence of 1249G>A on mRNA level. A trend of lower expression in carriers of the variant allele was observed in preterm placenta samples (n = 26). A similar, but not significant trend was also observed for 3972C>T associated with the MRP2 protein expression level in both preterm and full-term placentas (33). This observation needs further studies to evaluate the significance, possibly with larger sample sizes.

Organic anion transporting polypeptides (OATPs)

The OATPs belong to the superfamily of SLC, and the genes are classified in the *SLCO* family of genes. These transporters are important in the uptake transport of amphipathic physiological substrates in a sodium and ATP-independent manner, including bile salts, steroid conjugates and thyroid hormones into the syncytiotrophoblast, although their usage in the fetus is not known (5). OATP2B1 and OATP4A1 proteins are present in the syncytiotrophoblast, while only low levels of mRNAs were detected for OATP1A2, OATP1B1 and OATP1B3 in the placental trophoblast (38–41). OATP2B1 is localized on the basolateral surface of the syncytiotrophoblast and membranes of the cytotrophoblast (42) while OATP4A1 is predominantly found in the apical membrane of the syncytiotrophoblast (43).

Recent studies have reported 49 polymorphisms in the *SLCO1B1* gene (44,45) and 388A>G was the most common in most ethnicities. Relative to other populations, it was more abundant in the African population (60–80%) (46), while 521T>C was present in less than 22.5% of Caucasians, African–Americans and Asians. The most frequently occurring functional SNPs for the *SLCO1B3* gene were the 334T>G (Ser112Ala) and 699G>A (Met233Ile), which were present in the majority of the

Caucasian and Asian populations (**Appendix 3.1**).

Effect of polymorphisms in human placenta: substrate transport

Since the OATPs are not very abundant in the placenta, the effect of *SLCO* genes in drug transfer in this tissue is not yet widely studied. Only one study used the placental tissue to investigate the effects of three *SLCO* gene polymorphisms on the transfer of repaglinide (**Appendix 3.3**). The mRNAs of *SLCO1B1* and *SLCO1B3* genes have been found in the placenta, but not their proteins (OATP1B1 and OATP1B3), yet the results showed that a haplotype of the *SLCO1B3* gene caused changes in repaglinide transfer. Samples with the heterozygous haplotype 334T>G/699G>A (n = 4) showed higher fetal-to-maternal transfer of repaglinide compared with the wild-type haplotype, 334GG/699AA (n = 2) (p = 0.09) (41). None of the other SNPs identified in the placenta samples were found to be associated with significant changes in the repaglinide transfer (41). Although the sample size was extremely limited, this study provided an initial result on the effect of *OATP* polymorphisms in placental tissue, which necessitates further study in determining whether the same effect holds true for drug substrates other than repaglinide, preferably with larger sample sizes.

Other placental transporter proteins

The organic cation transporters (OCTs), organic cation/carnitine transporters (OCTN2) and multidrug and toxin extrusion transporters (MATEs) are also expressed in the placental tissue. Known polymorphisms of these transporters have been reported, but nothing has been reported regarding their effects in the placental tissue.

Among the OCTs, OCT3, known as the 'placenta- specific' OCT, is most abundant in the placenta and uterine tissue, compared with OCT1 and OCT2, which are predominantly expressed in the liver and kidney tissues, respectively. Encoded by the *SLC22A3* gene, it is localized at the basolateral membrane of the syncytiotrophoblast together with OCT1 (*SLC22A1*) (40). Transport of organic cations mediated by OCT3 in the placenta is electrogenic, independent of Na+, reversible with respect to direction and occurs in a concentration-dependent manner (47). This explains how this transporter is capable of transporting the maternally administered cationic compounds from the placenta to the fetal side and oppositely into the placenta when the level is higher in the fetal blood (48). It also presumably plays a role in the excretion of metabolic waste products or xenobiotics from the fetus. Sakata and colleagues have reported three SNPs which are functional *in vitro*: 346G>T, 1199C>T and 1316C>T, in which 346G>T was only present in African–Americans, and the latter two were observed in Asians and Caucasians (49). For OCT1, the relatively more common 1022C>T (Pro341Leu) and 1222A>G (Met480Val) were extensively

explored and responsible for the alterations in transporter function in relation to metformin response [50].

The OCTNs include OCTN1 (*SLC22A4*) and OCTN2 (*SLC22A5*), which are both structurally related to the OCTs, and are most abundant in kidney and placental tissues (47,51). Both are localized at the apical surface of the syncytiotrophoblast. They transport carnitine, an endogenous metabolite important for lipid metabolism, from the mother to the fetus through pH-dependent transport (52,53) as well as organic cations and cationic drugs (54). The most studied SNP of the *SLC22A4* gene, 1507C>T, was markedly present in Caucasians (39–43%) in contrast to African–Americans and Asians (55).

The MATEs are encoded by the *SLC47A* gene family, MATE1 by the *SLC47A1* and MATE2 by the *SLC47A2* gene. There are two splice variants found for MATE2: MATE2-K and MATE2-B, with the former being the active form and predominantly expressed. MATE1 is highly expressed in the kidney, adrenal gland, skeletal muscle and other tissues, while MATE2-K is a kidney-specific protein. Both function as the H+/organic cation antiporter in transporting the organic cations (56). Recently the expression of MATE1 and MATE2 mRNA was also observed in the first trimester human placenta, although with a high level of variability, while in the full-term placenta, MATE1 mRNA was not detected (57). A study in the rat fullterm placenta demonstrated that Mate1 was responsible for the efflux of organic cation into the maternal circulation after it was taken into the cell from the fetal circulation by Oct3 (58). Therefore it may indicate that the MATE1 is expressed at the apical membrane of the syncytiotrophoblast. MATEs transport organic cations including some drugs: metformin, cimetidine, procainamide, and some anionic and zwitterionic compounds (59). Polymorphisms of *SLC47A1* have been explored for a few SNPs that are only present in less than 3% of Asians (60,61).

Translating functional effects of polymorphisms into phenotype groups based on in vitro transport studies

In clinical studies, the polymorphisms of transporter proteins are generally investigated for their implications for the pharmacokinetic, clinical response and susceptibility for adverse drug reactions, which are likely to be associated with their role in regulating the drug level in the blood circulation (for extensive reviews see (62–65)). The effect of transporter polymorphisms on drug disposition may vary between tissues. Studies on pharmacokinetics and therapeutic efficacy of, for example paclitaxel, in association with P-gp polymorphisms showed different effects in different tissues. 3435C>T and 2677G>T/A have been shown to be associated with a reduction in paclitaxel clearance in renal tissues (66,67), but no such correlation was

found in other studies (68,69). Studies on the efficacy of paclitaxel have found that 2677G>T/A was beneficial in ovarian cancer patients treated with this drug (70), but no such association was noted in other studies (71,72).

The disparity of the results may have been caused by, among others, the substrate-dependent effect and the substrate-specificity effect. The substrate-dependent effect, as discussed earlier, takes into account the transport activity of other transporters. The second effect concerns the substrate specificity, in which multiple binding domains may be present for different substrates in one transporter protein. A nonsynonymous SNP causes changes in the amino acid sequence and protein conformation and can affect the binding site and transport kinetics of some drugs, while the efflux activity of other drugs with different binding sites is not affected (73). As the SNP causes alteration in substrate recognition, it is also likely to lead to changes in transporter function (74,75). For example, the 848C>T and 1022C>T variants of the *SLC22A1* gene encoding for OCT1, did not show altered metformin uptake compared with the wild-type variant, but reduced uptake of lamivudine was observed with both variants (76). The changes in substrate recognition may explain affinity changes in substrates, as seen in the OCTN2 -503F variant, which was associated with less affinity for carnitine and other endogenous substrates but higher affinity for tetraethylammonium and various xenobiotics (verapamil, cimetidine, lidocaine and quinidine) than the wild-type (77).

Apart from the substrate-dependent and substrate specificity effects, an interesting effect of haplotypes was also observed. One example is the effect of the 1222G>A/1258A>del (M420del/M408V) haplotype of the *SLC22A1* gene in the transport of imatinib. The 1258del variant itself reduces the transport activity of this drug, but no effect was found in haplotype 1222G/1258del, indicating that the presence of 1222G counteracts the effect of the 1258del variant (78). Haplotype studies may provide a better explanation and understanding of the functional effect of polymorphisms. 3435C>T has been shown to be functional despite the fact that it is a synonymous SNP, in which a haplotype effect was observed instead of an effect of the SNP itself.

The effect of transporter polymorphisms on the pharmacokinetic and pharmacodynamic parameters of the substrate drugs requires further understanding of other factors, including variation in the nature of transporter expression in different cells. Therefore, we take into account only the results from studies using transfected cell lines on the effect of polymorphisms in the *in vitro* transport activity of the placental transporters. These studies showed a more classical way to determine the direct effect of polymorphism, excluding the effect of other physiological

parameters or underlying mechanisms involved in *in vivo* studies.

Comparison and interpretation of the results is also challenging since there is no generally accepted method to perform this. In genetic association analysis, studies using rare SNPs with very low allele frequency may need a large sample size to conclude an association between the SNP and the outcome. We therefore propose to classify the phenotypes as either 'increased activity', 'decreased activity', 'normal' or 'abolished activity', based on the *in vitro* transport activity of drug substrates. If the information on drug substrates is unavailable, the classification will be based on the results of the endogenous substrate transport. Some of the functional SNPs listed in **Appendix 3.1** are not commonly found in the population, for example, 571G>A and 1292-3GT->TG, which are both found only in leukemia patients (79,80). Therefore, grouping the SNPs by similar functional *in vitro* effect, as has previously been done for metabolic enzymes such as CYP2D6 (81), will provide a reasonable option in genetic studies to search for associations between transporter polymorphisms and functional effect (**Table 1 & Appendix 3.4**). By grouping SNPs together according to their functionality, a cumulative phenotype frequency will be used instead of the allele frequency of individual SNPs. This allows for genetic association studies using a smaller sample size with reasonable effect sizes and power.

Do transporter protein polymorphisms play a role in the risk for drug-induced birth defects?

As transporter protein polymorphisms may influence the placental transfer of drugs, it is likely that the fetal exposure to drugs taken by the mother is affected. There are few studies exploring the effects of transporter protein polymorphisms on the outcome of the newborn, and even fewer focused specifically on the events of drug-induced birth defects. Earlier animal studies suggest that Mdr1a and Mdr1b genes, encoding for P-gp in mice, play a role in the protection of the fetus from teratogenic effect of xenobiotics in the maternal bloodstream, and the fetal genotype is shown to be important in regulating the protection (82,83). The mice strains with knockout P-gp genes have shown susceptibility to cleft palate following the administration of phenytoin to the pregnant dams. Among 48 fetuses with cleft palate, 36 had the heterozygous (mdr1a+/-) genotype and 12 were homozygous for the knockout gene (mdr1a-/-) (83).

Table 1. Summary of phenotype groupings of placental transporter polymorphisms according to *in vitro* transport of substrates.

Placental transporters	SNPs with phenotype groupings			
	Increased	**Normal**	**Decreased**	**Diminished**
P-gp	1236C>T†, 2677G>T/A†, 3435C>T†, 266T>C, 1985T>G, 1199G>A, 2005C>T, 3421T>A	61G>A, 3751G>A, 2677G>A	571G>A, 1199G>T, 1292-3GT>TG, 3322T>C	NA
MRP2	4430C>T	1249G>A, 4430C>T	2366C>T, 4348G>A, 4430C>T, 4544G>A	NA
OATP1B3	NA	767C>G†, 334T>G/699G>A†	NA	NA
OATP2B1	NA	-282G>A†, 935G>A†	NA	NA
OATP1B1	NA	388A>G†, 521T>C†	NA	NA
OCT1	NA	480C>G, 848C>T, 1022C>T, 1222A>G,1258A>del, 1222A>G/1258A>del	41C>T, 181C>T, 848C>T, 1022C>T, 289C>A, 350C>T, 616C>T, 566C>T, 659G>T, 1201G>A, 1258A>del, 1393G>A	848C>T, 859C>G
OCT3	NA	NA	346G>T, 1199C>T, 1316C>T	NA
OCTN1	1507C>T	NA	1507C>T	NA
OCTN2	-207G>C	430C>T, 1645C>T	-207G>C, 1441G>T,51C>G, 364G>T, 904A>G	NA
MATE1	1557G>C	373C>T, 1012G>A	191G>A, 1438G>A, 404T>C, 1012G>A, 191G>A, 929C>T, 983A>C, 1012G>A, 1421A>G, 1557G>C, 373C>T	191G>A, 1438G>A
MATE2	NA	NA	192G>T	632_633GC>TT

See Appendix 3.3 & Appendix 3.4 for further details.

† Transport activity through theplacenta. NA, not available

With regards to clinical studies, two studies have reported the role of P-gp 3435C>T in the risk of drug- induced birth defects. In contrast to the previous study, which demonstrates a role of the fetal genotype, these studies have shown that the maternal genotype is an important determinant of risk for birth defects. A study by Bliek et al. has shown that mothers carrying the 3435TT genotype and using any type of medication during the periconception period had a significantly higher risk (6.2-fold) of having children with cleft lip with or without cleft palate. The risk is increased to 19.2-fold for mothers who were not taking periconceptional folic acid supplementation (84). On the other hand, Obermann-Borst et al. have found an association between maternal 3435CT/ TT genotypes, maternal general medication use and an increased risk of congenital heart defects among the offspring, although the association was not statistically significant. Nevertheless, the risk was significantly increased to threefold in mothers who were not taking folic acid supplementation. Children who were carriers of 3435CT/TT genotypes, were also at an increased risk of having this defect compared with children with the CC genotype, and the risk was increased to threefold if the mothers were not taking folic acid supplementation. When both maternal and fetal genotypes were taken into consideration, the risk was fairly increased (85).

The results from Bliek et al. and Obermann-Borst et al. support the conclusion of Hitzl et al. that lower P-gp expression in the placenta was observed with the maternal 3435TT genotype (18), thus increasing the susceptibility of the fetus to teratogenicity. The results from the clinical studies discussed above suggest that transporter polymorphisms play a role in fetal drug exposure and subsequently the risk for drug-induced birth defects. Therefore, more studies are warranted to shed light on this issue. With regards to that, there are several determinants that need to be considered before performing similar types of research. The first determinant is the type of medications used by the mothers, which are suspected to cause birth defects. The medications that have strong associations with certain birth defects, and are also transported by the transporter of interest, are more likely to be relevant in the study. The second determinant is the source of genotype, either from the mother or the child. The genotype of the mother determines the pharmacokinetics of the drug in the maternal circulation, which later determines the level of fetal exposure to the drug. The fetal genotype, on the other hand, contributes to the transporter expression in the placenta. The best way to measure the association is to consider the genotypes from both the mother and the child.

There is an abundant room for further progress in determining the role of transporter polymorphisms in evaluating the risk for drug-induced birth defects. However, there are many challenges in executing pharmacogenetic studies in this topic. First of all,

maternal physiological changes during pregnancy may alter the pharmacokinetics of the drug of interest. With regards to the maternal drug circulation, the transporter proteins expressed in other major tissues, such as liver, kidney and intestine also play an important role in drug distribution and excretion from the maternal circulation. Efflux transporters expressed in the intestine may affect the oral bioavailability of drug substrates, while those that are expressed in the kidney and liver may have an effect on the hepatobiliary or renal excretion of drug substrates. Any changes in the expression and function of these transporters associated with the maternal genetic polymorphisms may also affect the drug level in the maternal circulation, which further leads to variation in substrate concentrations available for maternal-to-fetal transport.

The second challenge is that there is no direct way of measuring the transporter protein function and fetal exposure to drugs. Previous *in vivo* and *in vitro* studies have only been carried out using full-term placentas, even though the expression of transporter proteins is known to change throughout gestational age. It is not currently known whether polymorphisms may alter the protein expression in one stage but not the other. Therefore it is unclear whether the change in transporter function is different between the placenta of the first trimester and full-term placenta.

The next challenge is the role of metabolic enzymes in the pharmacokinetics of drugs, in which the enzymes are highly polymorphic. It is widely known that the transporter proteins and metabolic enzymes work together in a 'drug transporter-metabolism alliance' (as proposed by Benet (86)) by limiting the entry of teratogenic drugs into the fetus. Polymorphisms of either one or both of them may affect their functions, and these factors are difficult to measure. In addition, metabolic enzymes are also expressed by the placenta to further limit the entry of reactive drug metabolites that might be teratogenic (see review by Rubinchik-Stern and Eyal (87)).

The placental barrier itself is believed to be very protective for the fetus, and minor changes in transporter activity may be counteracted by other mechanisms of protection in a normal physiologically functional placenta. Moreover the same transporters may also be expressed in the fetal capillary endothelium as well as in the syncytiotrophoblast. Functionality changes associated with polymorphisms are conflicting for certain transporter proteins and some of the teratogenic drugs are transported by more than one transporter. Therefore, it is difficult to detect which polymorphisms of which transporters are responsible for the changes in risk of drug-induced birth defects.

CONCLUSION & FUTURE PERSPECTIVE

The mechanism of maternal-to-fetal drug transport is complex, substrate dependent and affected by physiological changes over the stages of gestation. Therefore, it is an intricate process to measure fetal drug exposure during pregnancy. New methods or study designs that can address the limitations mentioned in this review may be useful in exploring the role of transporter protein polymorphism in drug teratogenicity. One of the possibilities is to use the preterm placental tissue from therapeutic abortions, instead of full-term placenta, to mimic the physiological conditions during the first trimester of pregnancy. Another possibility is to perform retrospective observational studies with the occurrence of birth defects as the outcome. Cases of birth defects with a history of prenatal exposure to teratogenic drugs may provide a proxy for an increased fetal exposure to drugs, compared with nonmalformed controls. This design may provide knowledge on the teratogenic risks based on the interactions effect between genetic polymorphisms and exposure to drugs. Analysis of the SNPs using phenotype groupings instead of individual SNPs enables genetic association studies to be performed using smaller sample sizes. Birth defects are rare disorders, and finding the cases that have been exposed to drugs adds to the challenge. Nevertheless, it is intriguing to explore whether pharmacogenetics contributes to the risk of drug-induced birth defects, and specifically, why certain fetuses are more susceptible to the teratogenic effect of drugs than others.

This topic involves the health of not only pregnant women taking clinically indicated drugs, but also of the future generation. Further knowledge on patient susceptibility may guide healthcare providers in making risk–benefit assessments in the selection of drugs for these women. Hence we anticipate that more attention will be given to this field in the next few years or decades.

REFERENCES

1. Ganapathy V, Prasad PD. Role of transporters in placental transfer of drugs. Toxicol. Appl. Pharmacol. 207(2 Suppl.), 381–387 (2005).

2. Iqbal M, Audette MC, Petropoulos S, Gibb W, Matthews SG. Placental drug transporters and their role in fetal protection. Placenta 33(3), 137–142 (2012).

3. Vahakangas K, Myllynen P. Drug transporters in the human blood–placental barrier. Br. J. Pharmacol. 158(3), 665– 678 (2009).

4. Prouillac C, Lecoeur S. The role of the placenta in fetal exposure to xenobiotics: importance of membrane transporters and human models for transfer studies. Drug Metab. Dispos. 38(10), 1623–1635 (2010).

5. Staud F, Cerveny L, Ceckova M. Pharmacotherapy in pregnancy; effect of ABC and SLC transporters on drug transport across the placenta and fetal drug exposure. J. Drug Target. 20(9), 736–763 (2012).

6. Nava-Ocampo AA, Koren G. Human teratogens and evidence-based teratogen risk counseling: the Motherisk approach. Clin. Obstet. Gynecol. 50(1), 123–131 (2007).

7. Kerb R. Implications of genetic polymorphisms in drug transporters for pharmacotherapy. Cancer Lett. 234(1), 4–33 (2006).

8. Ieiri I. Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). Drug Metab. Pharmacokinet. 27(1), 85–105 (2012).

9. Ieiri I, Takane H, Hirota T, Otsubo K, Higuchi S. Genetic polymorphisms of drug transporters: pharmacokinetic and pharmacodynamic consequences in pharmacotherapy. Expert Opin. Drug Metab. Toxicol. 2(5), 651–674 (2006).

10. Ieiri I, Higuchi S, Sugiyama Y. Genetic polymorphisms of uptake (OATP1B1, 1B3) and efflux (MRP2, BCRP) transporters: implications for inter-individual differences in the pharmacokinetics and pharmacodynamics of statins and other clinically relevant drugs. Expert Opin. Drug Metab. Toxicol. 5(7), 703–729 (2009).

11. Sakaeda T, Nakamura T, Okumura K. Pharmacogenetics of drug transporters and its impact on the pharmacotherapy. Curr. Top. Med. Chem. 4(13), 1385–1398 (2004).

12. Hutson JR, Koren G, Matthews SG. Placental P-glycoprotein and breast cancer resistance protein: influence of polymorphisms on fetal drug exposure and physiology. Placenta 31(5), 351–357 (2010).

13. Schinkel AH, Jonker JW. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Adv. Drug Deliv. Rev. 55(1), 3–29 (2003).

14. Ni Z, Mao Q. ATP-binding cassette efflux transporters in human placenta. Curr. Pharm. Biotechnol. 12(4), 674– 685 (2011).

15. Mickley LA, Lee JS, Weng Z et al. Genetic polymorphism in MDR-1: a tool for examining allelic expression in normal cells, unselected and drug-selected cell lines, and human tumors. Blood 91(5), 1749–1756 (1998).

16. Hoffmeyer S, Burk O, von Richter O et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc. Natl Acad. Sci. USA 97(7), 3473–3478 (2000).

17. Takane H, Kobayashi D, Hirota T et al. Haplotype- oriented genetic analysis and functional assessment of promoter variants in the MDR1 (ABCB1) gene. J. Pharmacol. Exp. Ther. 311(3), 1179–1187 (2004).

18. Hitzl M, Schaeffeler E, Hocher B et al. Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). Pharmacogenetics 14, 309–318 (2004).

19. Rahi M, Heikkinen T, Hakkola J et al. Influence of adenosine triphosphate and ABCB1 (MDR1) genotype on the P-glycoprotein-dependent transfer of saquinavir in the dually perfused human placenta. Hum. Exp. Toxicol. 27(1), 65–71 (2008).

20. Hemauer SJ, Nanovskaya TN, Abdel-Rahman SZ, Patrikeeva SL, Hankins GD, Ahmed MS. Modulation of human placental P-glycoprotein expression and activity by MDR1 gene polymorphisms. Biochem. Pharmacol. 79(6), 921–925 (2010).

21. Tanabe M, Ieiri I, Nagata N et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. J. Pharmacol. Exp. Ther. 297(3), 1137–1143 (2001).

22. Molsa M, Heikkinen T, Hakkola J et al. Functional role of P-glycoprotein in the human blood-placental barrier. Clin. Pharmacol. Ther. 78(2), 123–131 (2005).

23. Rahi M, Heikkinen T, Härtter S et al. Placental transfer of quetiapine in relation to P-glycoprotein activity. J. Psychopharmacol. 21(7), 751–756 (2007).

24. Hahnova-Cygalova L, Ceckova M, Staud F. Fetoprotective activity of breast cancer resistance protein (BCRP, ABCG2): expression and function throughout pregnancy. Drug Metab. Rev. 43(1), 53– 68 (2011).

25. Mao Q. BCRP/ABCG2 in the placenta: expression, function and regulation. Pharm. Res. 25(6), 1244–1255 (2008).

26. Evseenko DA, Paxton JW, Keelan JA. Independent regulation of apical and basolateral drug transporter expression and function in placental trophoblasts by cytokines, steroids, and growth factors. Drug Metab. Dispos. 35(4), 595– 601 (2007).

27. Mathias AA, Hitti J, Unadkat JD. P-glycoprotein and breast cancer resistance protein expression in human placenta of various gestational ages. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289, R963–R969 (2005).

28. Yeboah D, Sun M, Kingdom J et al. Expression of breast cancer resistance protein (BCRP/ABCG2) in human placenta throughout gestation and at term before and after labor. Can. J. Physiol. Pharmacol. 84, 1251–1258 (2006).

29. Polgar O, Robey RW, Bates SE. ABCG2: structure, function and role in drug response. Expert Opin. Drug Metab.Toxicol. 4(1), 1–15 (2008).

30. Imai Y, Nakane M, Kage K et al. C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Mol. Cancer Ther. 1, 611–616 (2002).

31. Kobayashi D, Ieiri I, Hirota T et al. Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. Drug Metab. Dispos. 33(1), 94–101 (2005).

32. St-Pierre M V., Serrano MA, Macias RI. et al. Expression of members of the multidrug resistance protein family in human term placenta. Am. J. Physiol. Integr. Comp. Physiol. 279, R1495–R1503 (2000).

33. Meyer zu Schwabedissen HE, Jedlitschky G, Gratz M et al. Variable expression of MRP2 (ABCC2) in human placenta: influence of gestational age and cellular differentiation. Drug Metab. Dispos. 33(7), 896–904 (2005).

34. Ito S, Ieiri I, Tanabe M, Suzuki A, Higuchi S, Otsubo K. Polymorphism of the ABC transporter genes, MDR1, MRP1 and MRP2/cMOAT, in healthy Japanese subjects. Pharmacogenetics 11(2), 175–184 (2001).

35. Itoda M, Saito Y, Soyama A et al. Polymorphisms in the ABCC2 (cMOAT/MRP2) gene found in 72 established cell lines derived from Japanese individuals: an association between single nucleotide polymorphisms in the 5'-untranslated region and exon 28. Drug Metab. Dispos. 30(4), 363–364 (2002).

36. Ho WF, Koo SH, Yee JY, Lee JD. Genetic variations of the ABCC2 gene in the Chinese, Malay, and Indian populations of Singapore. Drug Metab. Pharmacokinet. 23(5), 385–391 (2008).

37. Haenisch S, May K, Wegner D, Caliebe A, Cascorbi I, Siegmund W. Influence of genetic polymorphisms on intestinal expression and rifampicin-type induction of ABCC2 and on bioavailability of talinolol. Pharmacogenet. Genomics. 18(4), 357–365 (2008).

38. Briz O, Serrano MA, MacIas RIR, Gonzalez-Gallego J, Marin JJ. Role of organic anion-transporting polypeptides, OATP-A, OATP-C and OATP-8, in the human placenta- maternal liver tandem excretory pathway for fetal bilirubin. Biochem. J. 371(Pt 3), 897–905 (2003).

39. Tamai I, Nezu J, Uchino H et al. Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. Biochem. Biophys. Res. Commun. 273(1), 251–260 (2000).

40. Klaassen CD, Aleksunes LM. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. Pharmacol. Rev. 62(1), 1–96 (2010).

41. Tertti K, Petsalo A, Niemi M et al. Transfer of repaglinide in the dually perfused human placenta and the role of organic anion transporting polypeptides (OATPs). Eur. J. Pharm. Sci. 44(3), 181–186 (2011).

42. Ugele B, St-Pierre M V, Pihusch M, Bahn A, Hantschmann P. Characterization and identification of steroid sulfate transporters of human placenta. Am. J. Physiol. Endocrinol. Metab. 284(2), E390–E398 (2003).

43. Sato K, Sugawara J, Sato T et al. Expression of organic anion transporting polypeptide E (OATP-E) in human placenta. Placenta. 24(2–3), 144–148 (2003).

44. Jada SR, Lim R, Wong CI et al. Role of UGT1A1*6, UGT1A1*28 and ABCG2 c.421C>A polymorphisms in irinotecan-induced neutropenia in Asian cancer patients. Cancer Sci. 98(9), 1461–1467 (2007).

45. Boivin A-A, Cardinal H, Barama A, Pichette V, Hébert M-J, Roger M. Organic anion transporting polypeptide 1B1 (OATP1B1) and OATP1B3: genetic variability and haplotype analysis in white Canadians. Drug Metab. Pharmacokinet. 25(5), 508–515 (2010).

46. Aklillu E, Mugusi S, Ngaimisi E et al. Frequency of the SLCO1B1 388A>G and the 521T>C polymorphism in Tanzania genotyped by a new LightCycler®-based method. Eur. J. Clin. Pharmacol. 67(11), 1139–1145 (2011).

47. Koepsell H, Endou H. The SLC22 drug transporter family. Pflugers Arch. 447(5), 666–676 (2004).

48. Sata R, Ohtani H, Tsujimoto M et al. Functional analysis of organic cation transporter

3 expressed in human placenta. J. Pharmacol. Exp. Ther. 315(2), 888–895 (2005).

49. Sakata T, Anzai N, Kimura T et al. Functional analysis of human organic cation transporter OCT3 (SLC22A3) polymorphisms. J. Pharmacol. Sci. 113(3), 263–266 (2010).

50. Shu Y, Sheardown SA, Brown C et al. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. J. Clin. Invest. 117(5), 1422–1431 (2007).

51. Klaassen CD, Aleksunes LM. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. Pharmacol. Rev. 62(1), 1–96 (2010).

52. Tamai I, Ohashi R, Nezu J et al. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J. Biol. Chem. 273(32), 20378–20382 (1998).

53. Yabuuchi H, Tamai I, Nezu J et al. Novel membrane transporter OCTN1 mediates multispecific, bidirectional, and pH-dependent transport of organic cations. J. Pharmacol. Exp. Ther. 289(2), 768–773 (1999).

54. Ohashi R, Tamai I, Yabuuchi H et al. Na(+)-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. J. Pharmacol. Exp. Ther. 291(2), 778–784 (1999).

55. Toh DS, Koo SH, Limenta LM, Yee JY, Murray M, Lee EJ. Genetic variations of the SLC22A4 gene in Chinese and Indian populations of Singapore. Drug Metab. Pharmacokinet. 24(5), 475–481 (2009).

56. Terada T, Inui K. Physiological and pharmacokinetic roles of H+/organic cation antiporters (MATE/SLC47A). Biochem. Pharmacol. 75(9), 1689–1696 (2008).

57. Ahmadimoghaddam D, Zemankova L, Nachtigal P et al. Organic cation transporter 3 (OCT3/SLC22A3) and multidrug and toxin extrusion 1 (MATE1/SLC47A1) transporter in the placenta and fetal tissues: expression profile and fetus protective role at different stages of gestation. Biol. Reprod. 88(3), 55 (2013).

58. Ahmadimoghaddam D, Hofman J, Zemankova L et al. Synchronized activity of organic cation transporter 3 (Oct3/Slc22a3) and multidrug and toxin extrusion 1 (Mate1/Slc47a1) transporter in transplacental passage of MPP+ in rat. Toxicol. Sci. 128(2), 471–481 (2012).

59. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K-I. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. Biochem. Pharmacol. 74(2), 359–371 (2007).

60. Kajiwara M, Terada T, Ogasawara K et al. Identification of multidrug and toxin extrusion (MATE1 and MATE2-K) variants with complete loss of transport activity. J. Hum. Genet. 54(1), 40–46 (2009).

61. Chen Y, Teranishi K, Li S et al. Genetic variants in multidrug and toxic compound extrusion-1, hMATE1, alter transport function. Pharmacogenomics J. 9(2), 127–136 (2009).

62. Kerb R. Implications of genetic polymorphisms in drug transporters for pharmacotherapy. Cancer Lett. 234(1), 4–33 (2006).

63. Leschziner GD, Andrew T, Pirmohamed M, Johnson MR. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. Pharmacogenomics J. 7(3), 154–179 (2007).

64. Cascorbi I, Haenisch S. Pharmacogenetics of ATP-binding cassette transporters and

clinical implications. Methods Mol. Biol. 596, 95–121 (2010).

65. Ieiri I. Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). Drug Metab. Pharmacokinet. 27(1), 85–105 (2012).

66. Fransson MN, Gre H, Litton J, Friberg LE. Influence of cremophor EL and genetic polymorphisms on the pharmacokinetics of paclitaxel and its metabolites using a mechanism-based model. Drug Metab. Dispos. 39(2), 247–255 (2011).

67. Gréen H, Söderkvist P, Rosenberg P et al. Pharmacogenetic studies of paclitaxel in the treatment of ovarian cancer. Basic Clin. Pharmacol. Toxicol. 104(2), 130–137 (2009).

68. Nakajima M, Fujiki Y, Kyo S et al. Pharmacokinetics of paclitaxel in ovarian cancer patients and genetic polymorphisms of CYP2C8, CYP3A4, and MDR1. J. Clin. Pharmacol. 45(6), 674–682 (2005).

69. Sissung TM, Mross K, Steinberg SM et al. Association of ABCB1 genotypes with paclitaxel-mediated peripheral neuropathy and neutropenia. Eur. J. Cancer. 42(17), 2893–2896 (2006).

70. Gréen H, Söderkvist P, Rosenberg P, Horvath G, Peterson C. mdr-1 single nucleotide polymorphisms in ovarian cancer tissue: G2677T/A correlates with response to paclitaxel chemotherapy. Clin. Cancer Res. 12(3 Pt 1), 854–859 (2006).

71. Marsh S, King CR, McLeod HL, Paul J, Gifford G, Brown R. ABCB1 2677G>T/A genotype and paclitaxel pharmacogenetics in ovarian cancer. Clin. Cancer Res. 12(13), 4127; author reply 4127–4129 (2006).

72. Ludwig AH, Kupryjañczyk J. Does MDR-1 G2677T/A polymorphism really associate with ovarian cancer response to paclitaxel chemotherapy? Clin. Cancer Res. 12(20 Pt 1), 6204 (2006).

73. Woodahl EL, Ho RJY. The role of MDR1 genetic polymorphisms in interindividual variability in P-glycoprotein expression and function. Curr. Drug Metab. 5(1), 11–19 (2004).

74. Shu Y, Leabman MK, Feng B et al. Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. Proc. Natl Acad. Sci. USA 100(10), 5902–5907 (2003).

75. Takeuchi A, Motohashi H, Okuda M, Inui K. Decreased function of genetic variants, Pro283Leu and Arg287Gly, in human organic cation transporter hOCT1. Drug Metab. Pharmacokinet. 18(6), 409–412 (2003).

76. Choi MK, Song IS. Genetic variants of organic cation transporter 1 (OCT1) and OCT2 significantly reduce lamivudine uptake. Biopharm. Drug Dispos. 33(3), 170–178 (2012).

77. Peltekova VD, Wintle RF, Rubin LA et al. Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat. Genet. 36(5), 471–475 (2004).

78. Giannoudis A, Wang L, Jorgensen AL et al. The hOCT1 SNPs M420del and M408V alter imatinib uptake and M420del modifies clinical outcome in imatinib-treated chronic myeloid leukemia. Blood. 121(4), 628– 637 (2013).

79. Yang Z, Wu D, Bui T, Ho RJ. a novel human multidrug resistance gene MDR1 variant G571A (G191R) modulates cancer drug resistance and efflux transport. J. Pharmacol. Exp. Ther. 327(2), 474–481 (2008).

80. Crouthamel MH, Wu D, Yang Z, Ho RJY. A novel MDR1 GT1292–3TG (Cys431Leu)

genetic variation and its effect on P-glycoprotein biologic functions. AAPS J. 12(4), 548–555 (2010).

81. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin. Pharmacol. Ther. 83(2), 234–242 (2008).

82. Lankas GR, Wise LD, Cartwright ME, Pippert T, Umbenhauer DR. Placental P-glycoprotein deficiency enhances susceptibility to chemically induced birth defects in mice. Reprod. Toxicol. 12(4), 457– 463 (1998).

83. Rawles LA, Acuna D, Erickson RP. The role of multiple drug resistance proteins in fetal and/or placental protection against teratogen-induced orofacial clefting. Mol. Reprod. Dev. 74, 1483–1489 (2007).

84. Bliek BJ, van Schaik RHN, van der Heiden IP et al. Maternal medication use, carriership of the ABCB1 3435C > T polymorphism and the risk of a child with cleft lip with or without cleft palate. Am. J. Med. Genet. A. 149A(10), 2088–2092 (2009).

85. Obermann-Borst SA, Isaacs A, Younes Z et al. General maternal medication use, folic acid, the MDR1 C3435T polymorphism, and the risk of a child with a congenital heart defect. Am. J. Obstet. Gynecol. 204(3), 236. e1–236. e8 (2011).

86. Benet LZ. The drug transporter-metabolism alliance: uncovering and defining the interplay. Mol. Pharm. 6(6), 1631–1643 (2009).

87. Rubinchik-Stern M, Eyal S. Drug interactions at the human placenta: what is the evidence? Front. Pharmacol. 3, 1–7 (2012).

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PHARMACOGENETIC PREDICTORS ASSOCIATED WITH THE RISK OF DRUG TERATOGENICITY

- Knowledge and attitude regarding pharmacogenetics among formerly pregnant women in the Netherlands and their interest in pharmacogenetic research
- The risk of congenital heart anomalies following prenatal exposure to serotonin reuptake inhibitors – Is pharmacogenetics the key?
- Prenatal exposure to serotonin reuptake inhibitors and congenital heart anomalies: An exploratory gene-environment interaction study

chapter 5

Knowledge and attitude regarding pharmacogenetics among formerly pregnant women in the Netherlands and their interest in pharmacogenetic research

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ABSTRACT

Background: Pharmacogenetics is an emerging field currently being implemented to improve safety when prescribing drugs. While many women who take drugs during pregnancy would likely benefit from such personalized drug therapy, data is lacking on the awareness towards pharmacogenetics among women. We aim to determine the level of knowledge and acceptance of formerly pregnant women in the Netherlands regarding pharmacogenetics and its implementation, and their interest in pharmacogenetic research.

Methods: A population-based survey using postal questionnaires was conducted among formerly pregnant women in the Northern parts of the Netherlands. A total of 986 women were invited to participate.

Results: Of 219 women who returned completed questionnaires (22.2% response rate), only 22.8% had heard of pharmacogenetics, although the majority understood the concept (64.8%). Women who had experience with drug side-effects were more likely to know about pharmacogenetics [OR=2.06, 95% CI 1.16, 3.65]. Of the respondents, 53.9% were positive towards implementing pharmacogenetics in their future drug therapy, while 46.6% would be willing to participate in pharmacogenetic research. Among those who were either not willing or undecided in this regard, their concerns were about the consequences of the pharmacogenetic test, including the privacy and anonymity of their genetic information.

Conclusion: The knowledge and attitude regarding the concept of pharmacogenetics among our population of interest is good. Also, their interest in pharmacogenetic research provides opportunities for future research related to drug use during pregnancy and fetal outcome.

INTRODUCTION

Up to eight in ten pregnant women in developed countries take prescription drugs—excluding prenatal supplements and vitamins—at some time during their pregnancy (1-3). Pregnancy can be a crucial period for these women, as their drug efficacy and the risk of side-effects may change. Various factors influence the pharmacokinetics of drugs during pregnancy, including both physiological changes and genetic factors (4-6). Hence, in order to personalize drug therapy for pregnant women it is imperative that this process incorporates pharmacogenetics, i.e. knowledge of the genetic factors involved in drug pharmacokinetics and drug prescribing. Such personalization is especially important when managing pharmacotherapy in pregnant women, since pre-registration trials have not yet studied the risks of drugs in this high-risk group.

Although the implementation of pharmacogenetics in drug prescribing is already underway, it is important to ensure that the public is aware and understands this new concept. In particular, they first need to allow doctors to do pharmacogenetic testing before being prescribed certain drugs that are subjected to dose changes due to patient variability. According to one survey in Denmark, the level of background knowledge on pharmacogenetics among the general public is low (14.1%) (7), although the majority of participants in other studies conducted in Australia and the USA had a positive attitude towards the concept (8-10). However, the knowledge and attitude regarding pharmacogenetics specifically among pregnant women who also need to take their unborn child into account in drug therapy, has not yet been reported.

We therefore aimed to determine the level of knowledge of pharmacogenetics among formerly pregnant women in the Netherlands and identify the potential determinants affecting their knowledge and attitude towards this concept. Since pharmacogenetics is a relatively new field with regard to drug therapy during pregnancy, it cannot be implemented in a clinical setting until a suitable framework is in place. Such a framework requires the results of observational pharmacogenetic studies, i.e. gene-environment interaction studies, an increasing number of which are being conducted (11-14). Therefore, we also explored the interest of formerly pregnant women to participate in future studies on pharmacogenetics.

METHODS

Study design and settings

A population-based questionnaire was conducted between November 2015 and January 2016 to assess the knowledge and attitude of formerly pregnant women regarding pharmacogenetics. The study population included women who had been pregnant and had a history of medication use at some time in their lives. These women were identified from the University of Groningen IADB.nl pharmacy prescription database. IADB.nl contains the pharmacy data of approximately 600,000 people in several Dutch provinces, provided by 60 community pharmacies. The prescription rates of the IADB.nl population are representative of the population in the Netherlands (15). Our study population was retrieved from a pregnancy subset of IADB.nl, based on the linkage between a child and a woman who was 15 to 50 years older than the child and who shared the same home address (16,17). Details of this linkage and of its validation have been reported previously (18).

Participants

In November 2015, we selected participants who had given birth between 1 January 2011 and 31 December 2014. This resulted in 3689 eligible women who were registered over 37 pharmacies. Power analysis revealed that reporting of associations between the dependent variables ('knowledge', 'attitude' and 'interest') and determinants as independent variables would require a total of 135 respondents. This calculation was based on an odds ratio of 2, an effect size of 0.3, 80% power and 5% significance. As we estimated the response rate at 15%, we selected approximately 1000 women whom we sent the invitation letters.

We first selected community pharmacies that registered the highest number of eligible women. These pharmacies were invited to participate in the study by e-mail and/or telephone. For each participating pharmacy, we used the list of eligible women from the pregnancy subset of IADB.nl to select participants at random. We continued to invite the pharmacies until we reached 1000 eligible women. For these women, we obtained their latest address from the pharmacy and sent them a package containing an invitation letter, a questionnaire coded with a unique identification number, and a pre-paid return envelope.

Questionnaire

The questionnaires were in Dutch and divided into five sections: a) personal information; b) experiences with diseases and drug use; c) knowledge on

pharmacogenetics; d) attitude towards pharmacogenetics; and e) interest to participate in pharmacogenetic research. The internal validity of the questionnaire was assessed by a methodological advisor and members of the research group, and some questions were subsequently revised to be more precise and concise. The first draft of the questionnaire was tested on a pilot group of 25 women who had a mean age of 26 years. They were sent either a paper version or an electronic version of the questionnaire, together with accompanying questions aimed at assessing their understanding of the content and its clarity; the questionnaire was then revised accordingly.

The questions were mostly closed ended, with the options of 'Yes/No/I don't know'. The respondents were allowed to leave blank answers. Since the concept of pharmacogenetics is new to many, we provided a description of this term in the invitation letter and in section c), after a series of questions to measure their background knowledge. The description was as follows: *'Pharmacogenetics looks at the influence of your genetic traits on the effect of medication. It is possible that different people break down medication differently due to variations in their genetic traits'.* Although pharmacogenetics is one of the subject areas within personalized medicine, we do not introduce the broader terms ('personalized medicine', 'precision medicine', 'individualized medicine') in the questionnaire. It is because we focused on the relation between genetics and medication effects, and not the genetic factors related to medical diagnosis, therapy options, disease risk determination, etc.

Statistical analysis

For statistical analysis, we grouped the level of education reported by respondents into three levels: low (primary school, lower general secondary education, and lower vocational education), middle (higher general secondary education and intermediate vocational education), and high (higher vocational education and university). The experiences with medication use and the responses to questions on attitude and willingness were reported as frequency and percentages. We used multivariable logistic regression with complete case analysis to analyze associations between potential determinants (i.e. age, educational level, experiences with medication use) and sum scores in terms of the dependent variables (i.e. knowledge of the concept of pharmacogenetics, attitude towards pharmacogenetics).

Associations were expressed as odds ratios (OR) with 95% confidence intervals. 'Knowledge' and 'attitude' were analyzed by combining the responses to three questions to give a sum score. Respondents who answered 'Yes' to all three questions assessing knowledge were considered to have good knowledge of the concept of

pharmacogenetics. Respondents who answered 'Yes' to all three questions assessing their attitude were considered to have a good attitude towards the implementation of pharmacogenetics. The variable 'interest to participate in pharmacogenetic research' was analyzed either as "Yes" or "No/I don't know". Analyses were performed using PSAW Statistics, Version 22 (IBM Corporation, Armonk, NY, USA).

RESULTS

Respondents' characteristics

We contacted thirteen community pharmacies that had the highest number of eligible women, according to the IADB.nl database. Eight of these pharmacies agreed to participate in the study. Letters were successfully delivered to 986 selected participants, and 219 (22.2%) returned a completed questionnaire. The sampling method is depicted in **Figure 1** and the general characteristics and medical/medication history of the respondents are shown in **Table 1**. The mean age of the respondents was 34 years, which is older than that of all eligible women in the database (N=3689, mean=29 years, p <0.001). The majority of the respondents had an education level that was middle (46.1%) or high (44.3%). 22 (10%) of the respondents were pregnant at the time they filled in the questionnaire.

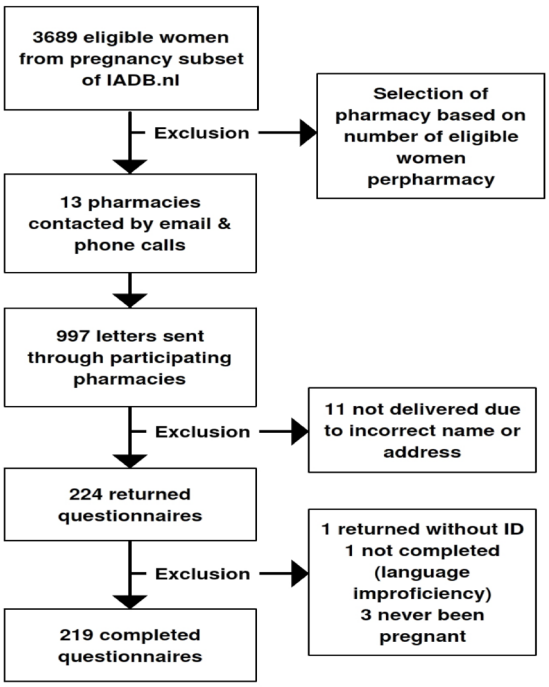

Figure 1: Population sampling and data collection methods

Table 1: General characteristics and medical/medication history of the respondents (N=219)

Characteristics	Respondents, n (%)
Age, mean years (range)	34 (25-46)
Education level	
Low	18 (8.2)
Middle	101 (46.1)
High	97 (44.3)
Living situation	
Alone/divorced	10 (4.6)
Married/living with partner/parents/others	207 (94.5)
History of chronic disease	
Yes	55 (25.1)
Never	116 (53.0)
No, but a family member has	47 (21.5)
History of medication use during pregnancy*	
Yes	94 (42.9)
No	119 (54.3)
Do not know	5 (2.3)
History of experiencing side-effects of medication	
Yes	93 (42.5)
No	105 (47.9)
No, but a family member has	13 (5.9)
Do not know	6 (2.7)
History of stopping medication use due to side-effects	
Yes	71 (32.4)
No	142 (64.8)
No, but a family member has	4 (1.8)
History of stopping medication use due to inefficacy	
Yes	52 (23.7)
No	160 (73.1)
No, but a family member has	5 (2.3)
Aware of the term 'pharmacogenetics'	
Yes	50 (22.8)
No	166 (75.8)
Aware of the meaning of 'pharmacogenetics'	
Yes	38 (17.4)
No	178 (81.3)

*excluding folic acid and other supplements

Fifty five (25.1%) of the respondents had one or more chronic diseases, mostly asthma, allergy and skin conditions. Of 163 who did not have a chronic disease, 47 (28.8%) had one or more family members with at least one chronic disease. One participant did not answer this question. Nearly half of all respondents had used some medication during pregnancy, excluding folic acid and vitamin supplements (n=94, 42.9%). A history of experiencing drug side-effects, not limited to the period of pregnancy, was also common among the respondents (n=93, 42.5%). Furthermore, 70% of this subset of respondents admitted to stopping their medication due to side-effects. Among all respondents, 23.7% (n=52) claimed that they had stopped taking medication due to ineffectiveness.

Knowledge of pharmacogenetics

A large percentage of the respondents were aware of the concept of pharmacogenetics (**Figure 2A**). 142 (64.8%) answered positively to all three questions that assessed their background knowledge on pharmacogenetics. However, only 50 (22.8%) of the respondents had heard the term 'pharmacogenetics' before receiving this questionnaire, while 38 (17.4%) of them claimed that they knew its meaning. Less than 10% of the respondents had heard of a 'DNA passport', which is personalized identification card containing genetic information to improve drug prescribing (DNA passports are commercially available in the Netherlands). Three respondents described the DNA passport correctly, while nine described it as containing an individual's genetic information in a broader scope, without mentioning the relation to drug use. Only one respondent had had a pharmacogenetic test, while five others knew someone who had had such a test.

Respondents with a high or medium level of education were more likely to agree with the statement "Do you think that there could be differences between people in their body's response to medication due to differences in their DNA?", than were respondents with a low level of education ([OR for middle level of education = 4.84, 95% CI 1.56, 15.0]; [OR for high level = 8.10, 95% CI 2.39, 27.51]) (**Table 2**). Respondents who had experience with drug side-effects, either themselves or their family members, were twice as likely to have a good knowledge of the pharmacogenetics concept [OR=2.06, 95% CI 1.16, 3.65]. Self-reported awareness of the term 'pharmacogenetics' and of the meaning of this term were not, however, associated with a good knowledge of pharmacogenetics.

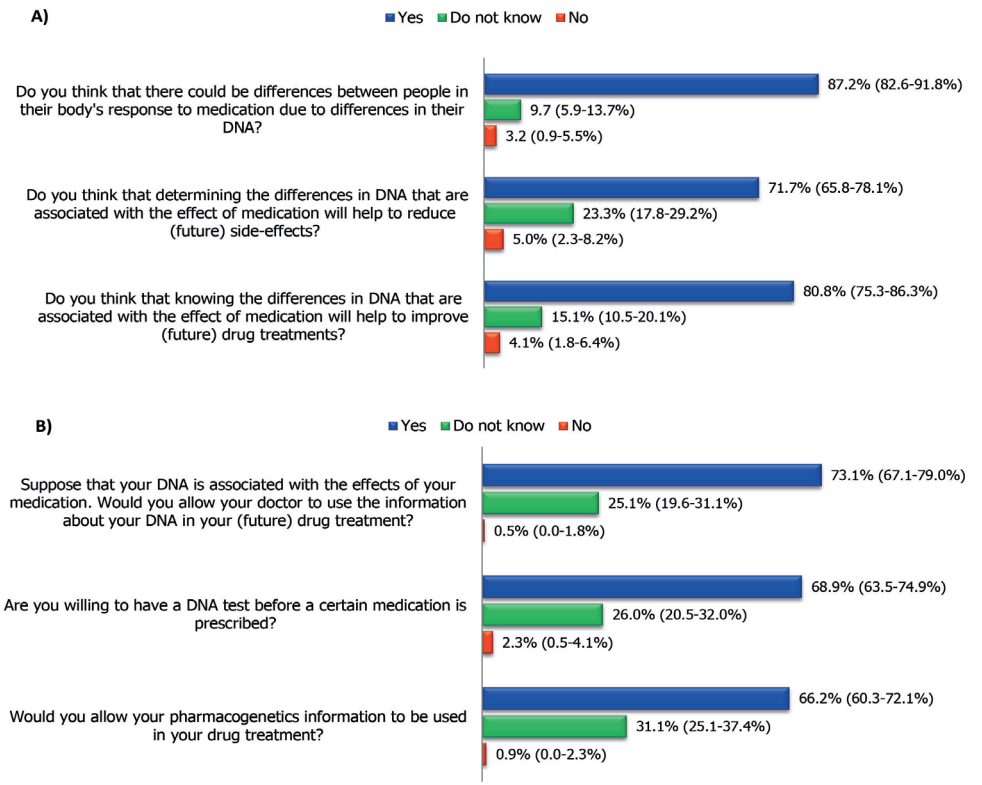

Figure 2: Knowledge and attitude towards pharmacogenetics among respondents (n=219).

A) The responses to the questions assessing their background knowledge of the concept of pharmacogenetics;
B) The responses to the questions assessing their attitude towards the implementation of pharmacogenetics

Table 2: The likelihood of having good knowledge about the concept of pharmacogenetics given respondent characteristics

Characteristics	Gave positive answers to knowledge questions, adjusted OR (95% CI)*, p value			
	Do you think that there could be differences between people in their body's response to medication due to differences in their DNA?	Do you think that determining the differences in DNA that are associated with the effect of medication will help to reduce (future) side-effects?	Do you think that knowing the differences in DNA that are associated with the effect of medication will help to improve (future) drug treatments?	Answering 'Yes' to all three questions (sum score of 3)
Educational level				
Low	Reference level			
Middle	**4.84 (1.56-15.0), 0.006**	1.08 (0.37-3.18), 0.88	1.34 (0.43-4.16), 0.62	1.62 (0.59-4.45), 0.35
High	**8.1 (2.39-27.51), 0.001**	1.65 (0.55-4.96), 0.37	2.69 (0.81-8.91), 0.11	2.57 (0.92-7.18), 0.07
Living situation (Living with spouse/ partner/others vs. alone/divorced)	0.74 (0.09-6.07), 0.78	0.56 (0.12-2.76), 0.56	1.02 (0.21-5.02), 0.98	0.75 (0.19-3.0), 0.75
Having chronic disease(s)† (Yes vs. No)	0.91 (0.36-2.32), 0.85	1.11 (0.55-2.23), 0.77	1.04 (0.47-2.32), 0.92	1.41 (0.72-2.875), 0.32
Having chronic disease(s)†† (Yes vs. No)	0.88 (0.38-2.0), 0.75	1.47 (0.80-2.71), 0.21	1.27 (0.63-2.56), 0.50	1.70 (0.96-3.02), 0.071
Used medication during pregnancy (Yes vs. No/Do not know)	0.99 (0.44-2.26), 0.99	1.62 (0.88-2.96), 0.12	1.40 (0.70-2.79), 0.34	1.23 (0.70-2.18), 0.47
Experienced side effect† (Yes vs. No/Do not know)	1.97 (0.82-4.74), 0.13	1.45 (0.79-2.67), 0.23	**2.13 (1.02-4.45), 0.045**	1.65 (0.93-2.94), 0.09
Experienced side effect†† (Yes vs. No/Do not know)	**2.66 (1.11-6.40), 0.029**	1.74 (0.95-3.20), 0.073	**2.56 (1.24-5.29), 0.011**	**2.06 (1.16-3.65), 0.014**
Stopping medication due to side effect(s) (Yes vs. No/do not know)	0.98 (0.42-2.32), 0.97	1.30 (0.68-2.48), 0.43	1.08 (0.52-2.24), 0.84	1.47 (0.80-2.71), 0.22
Stopping medication due to inefficacy (Yes vs. No/Do not know)	1.10 (0.42-2.90), 0.85	1.06 (0.52-2.14), 0.88	0.60 (0.28-1.27), 0.18	0.97 (0.50-1.88), 0.93
Aware of the term 'pharmacogenetics'	4.08 (0.93-17.91), 0.063	1.37 (0.64-2.93), 0.42	1.79 (0.70-4.58), 0.22	1.57 (0.77-3.19), 0.22
Aware of the meaning of 'pharmacogenetics'	2.88 (0.65-12.75), 0.16	1.53 (0.65-3.60), 0.33	3.01 (0.87-10.36), 0.081	1.80 (0.80-4.07), 0.16

*adjusted for age; †themselves; ††themselves or family members; bold font indicates significant associations.

Attitude towards pharmacogenetics

More than half of the respondents had a positive attitude towards pharmacogenetics (**Figure 2B**). 118 (53.9%) answered positively to all three questions assessing their attitude towards the implementation of pharmacogenetics in any drug treatments that they may need in the future. 20- 30% of the respondents were unsure of whether they would take a DNA test or would allow their doctors to use their pharmacogenetic information in their drug treatment. The most preferred method of DNA collection, voted as the first choice, was buccal swab collection (57.8%), followed by saliva collection (25.4%), dried blood spot (15%), and the least preferred method being taking a blood sample (1.2%) (**Appendix 4.1**). A positive attitude towards the implementation of pharmacogenetics was associated with having good knowledge of pharmacogenetics [OR=3.50, 95% CI 1.95, 6.30]. Other variables were not found to be significantly correlated with a positive attitude towards pharmacogenetics (**Appendix 4.2**).

Interest to participate in pharmacogenetic research

We determined respondents' interest to take part in future pharmacogenetic research: 102 (46.6%) were positive, 31 (14.2%) answered 'No', while 85 (38.8%) answered 'Do not know'. Among those who responded 'No' or 'Do not know' (n=116), the reason most often given for not wanting to participate in such research was that they were worried about the consequences (35.3%), while some of them did not want their DNA or genetic information to be used for research (12.9%). Many of them also described their concerns in the open-ended option provided and some of these are listed in **Table 3**. In addition, nearly half of the respondents were interested in obtaining more information on pharmacogenetics (n=98, 44.7%), while the percentages of those who declined or who were undecided were almost equal (23.7% and 29.2%, respectively). The preferred source of information was the internet (n=103, 47.0%), followed by an information leaflet (n=91, 41.6%), advice from their general practitioner (n=90, 41.1%), and advice from a pharmacist (n=41, 18.7%).

Good knowledge of the concept of pharmacogenetics and a positive attitude towards its implementation were significantly associated with the interest to participate in pharmacogenetic research [OR for 'knowledge' = 2.05, 95% CI 1.15, 3.66; OR for 'attitude' = 5.73, 95% CI 3.16, 10.37]. Women who had good knowledge and a positive attitude were also more likely to be interested in obtaining more information about the concept [OR for 'knowledge' = 1.85, 95% CI 1.03, 3.32; OR for 'attitude' = 2.25, 95% CI 1.29, 3.92].

Table 3: Reasons not to participate in pharmacogenetic research (N=116)

Reasons	n	%*
'I am worried about the consequences'	41	35.3
'I do not want my DNA/genetic information to be used in research'	15	12.9
'I am not interested in pharmacogenetic research'	10	8.6
'I do not understand the benefit of genetic testing'	8	6.9
Others (answers in open-ended option):		
'Need more information about the research before I can decide'	2	19.0
'Concerned about the privacy/anonymity of my genetic information and afraid it will be used/abused by insurance company, employer, etc.'	14	12.1
'Need more time to think about this'	5	4.3
'Lack of understanding or doubtful about this concept'	3	2.6
'Do not like/no time to participate in research'	2	1.7
Other/personal reasons	5	4.3

*percentages may add up to more than 100 because respondents could give more than one answer

DISCUSSION

This study suggests that while most women who have been pregnant are not familiar with the term 'pharmacogenetics', many do have some understanding of the association between DNA and drug therapy effects. Our results also show that while more than half of formerly pregnant women are positive towards the use of their genetic information in determining their future drug therapy, many of them are either neutral or still undecided.

Predictors of good knowledge and attitude towards pharmacogenetics

We found educational level to be one of the predictors of adequate knowledge on pharmacogenetics, while the majority of the respondents had at least a medium level of education. The high percentage of highly educated women among our respondents (44.3%) is similar to the percentage reported by Statistics Netherlands in 2011 for women aged between 25 and 34 years (43%) (19). The percentage of respondents who reported they had used medication during pregnancy (42.9%) is lower than the percentage of the source population who had received any prescription during pregnancy (71.4%, N=3689), and also lower than the percentage reported previously

in the same population (69.2%) (16). We expect that the actual drug use during pregnancy in our respondents was higher, since our data is based on self-reported events and subject to recall bias.

Our finding that respondents who had experienced drug side-effects themselves—or who had family members with side-effects—were more likely to have a good understanding and knowledge of the pharmacogenetics concept is consistent with the findings of Nielsen and colleagues (7). Others have also found a history of side-effects to be a good motivation for pharmacogenetic testing (10,20), even if the individual is aware of the possibility of the DNA sample being accessed without the patient's permission or if the sampling requires a blood test (10).

Women in our study population were generally positive and optimistic regarding the use of pharmacogenetics information for their future drug therapy. This attitude towards pharmacogenetics was not influenced by education level, chronic disease or a history of side-effects, but was significantly associated with a good understanding of the concept. The fact that when answering questions about their attitudes many respondents indicated they were undecided or unsure—rather than giving a negative answer—is not surprising. After all, a large majority of them might only have heard of pharmacogenetics for the first time from the questionnaire in this study, which may well explain their conservative opinions. Other reasons that might explain the reluctance in accepting pharmacogenetics are the lack of understanding or belief that this concept could help in their drug treatment, or they might be worried about the consequences (20,21).

Our results suggest that neither a history of chronic diseases nor medication use during pregnancy leads to a more positive attitude or perception on pharmacogenetics. In addition, the number of medications consumed also did not determine the attitude towards pharmacogenetics among the public (7). This finding shows that the concept of pharmacogenetics can be universally accepted by the public, regardless of health status and disease burden; we predict the public may well accept the use of pharmacogenetics as long as they are well informed of its possibilities.

Interest in pharmacogenetic research

In our study, the respondents' preferred sources of information about pharmacogenetics were different to those found in other studies. While we found the internet and an information leaflet to be most favored, others report the advice from doctors, a specialist or other healthcare providers to be more popular (20,22). One of the possible explanations is that we provided a link to a reliable website on pharmacogenetics in the questionnaire, which might aid their decision to choose

the internet. Regardless of their choice, our questionnaire itself might well stimulate participants' interest in finding out more about pharmacogenetics. Indeed, better knowledge on the concept of pharmacogenetics, and a positive attitude towards the implementation of pharmacogenetics were associated with a high interest to participate in future pharmacogenetic research. Concerns reported here such as those regarding the privacy and anonymity of genetic information —and possible misuse by employers or insurance companies—have also been reported by others (8,20,23,24). This emphasizes the need for regulatory measures to be established to protect patients' privacy.

There are three main limitations to our study. First, sampling bias may have arisen during the recruitment of community pharmacies, since we could only select those pharmacies registered in the pregnancy subset of IADB.nl, and several pharmacists declined to participate because the topic was deemed too difficult for their population. Second, information bias may also have affected our results in terms of respondents' knowledge about pharmacogenetics. Both the cover letter and the questionnaire provided respondents with a brief explanation of pharmacogenetics. While this information was intended to introduce this concept to those who were not familiar with it, and to promote interest in filling in the questionnaire (22,23), it may have assisted respondents in answering the questions, and resulted in a higher level of knowledge being reported. Third, there was a selection bias towards women who were more health-literate, and away from those who chose not to respond to the questionnaire. Although our survey results may not represent the entire Dutch female population, they do focus on a population who might be pregnant, and provide a useful picture of their views on pharmacogenetics and its implementation in their drug therapy.

A key strength of our study is that we are the first to report on the knowledge and attitude regarding pharmacogenetics specifically in a formerly pregnant population. While awareness of pharmacogenetics itself appears to be low, women's knowledge and attitude on the concept is good. Their acceptance and concerns regarding pharmacogenetic issues do not appear to be very different from those among the public and patient population as reported elsewhere (7,8,10,20,22). Future research in this area could include longitudinal studies of pregnant women and also exploring attitudes to DNA testing for pharmacogenetic information, before, during and after pregnancy.

CONCLUSION

The substantial level of interest in pharmacogenetics among formerly pregnant women is encouraging; therefore it is worthwhile pursuing its application in personalized drug therapy for safer use of drugs during pregnancy. Several designs in genetic epidemiology research may pave ways to understand the role of pharmacogenetics in fetal drug exposure and outcome. A question remained unanswered is whether the privacy and confidentiality of the genetic information can be adequately assured, in both research and clinical settings, which need to be addressed by researchers and health authorities.

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REFERENCES

1. Mitchell AA, Gilboa SM, Werler MM, Kelley KE, Louik C, Hernández-Díaz S. Medication use during pregnancy, with particular focus on prescription drugs: 1976-2008. Am. J. Obstet. Gynecol. 2011;205:51.e1-51.e8.

2. Daw JR, Hanley GE, Greyson DL, Morgan SG. Prescription drug use during pregnancy in developed countries : a systematic review. Pharmacoepidemiol. Drug Saf. 2011;20:895–902.

3. Cleary BJ, Butt H, Strawbridge JD, Gallagher PJ, Fahey T, Murphy DJ. Medication use in early pregnancy-prevalence and determinants of use in a prospective cohort of women. Pharmacoepidemiol. Drug Saf. 2010;19:408–17.

4. Isoherranen N, Thummel KE. Drug metabolism and transport during pregnancy : How does drug disposition change during pregnancy and what are the mechanisms that cause such changes ? Drug Metab. Dispos. 2013;41:256–62.

5. Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H. Anatomical, physiological and metabolic changes with gestational age during normal pregnancy. Clin. Pharmacokinet. 2012;51:365–96.

6. Haas DM. Obstetric therapeutics-How pharmacogenetics may inform drug therapy for pregnant women in the future. Obstet. Gynecol. Surv. 2013;68:650–4.

7. Nielsen LF, Moldrup C. The diffusion of innovation: Factors influencing the uptake of pharmacogenetics. Community Genet. 2007;10:231–41.

8. Haddy CA, Ward HM, Angley MT, McKinnon RA. Consumers' views of pharmacogenetics--A qualitative study. Res. Social Adm. Pharm. 2010;6:221–31.

9. Shaw JL, Robinson R, Starks H, Burke W, Dillard DA. Risk, reward, and the double-edged sword: perspectives on pharmacogenetic research and clinical testing among Alaska Native people. Am. J. Public Health. 2013;103:2220–5.

10. Haga S, O'Daniel J, Tindall G, Lipkus I, Agans R. Survey of U.S. public attitudes towards pharmacogenetic testing. Pharmacogenomics J. 2012;12:197–204.

11. Haas DM, Lehmann AS, Skaar T, Philips S, McCormick CL, Beagle K, et al. The impact of drug metabolizing enzyme polymorphisms on outcomes after antenatal corticosteroid use. Am. J. Obstet. Gynecol. 2012;206:e17-24.

12. Haas DM, Dantzer J, Lehmann AS, Philips S, Skaar TC, McCormick CL, et al. The impact of glucocorticoid polymorphisms on markers of neonatal respiratory disease after antenatal betamethasone administration. Am. J. Obstet. Gynecol. 2013;208:1–6.

13. Bliek BJB, van Schaik RHN, van der Heiden IP, Sayed-Tabatabaei FA, van Duijn CM, Steegers EAP, et al. Maternal medication use, carriership of the ABCB1 3435C > T polymorphism and the risk of a child with cleft lip with or without cleft palate. Am. J. Med. Genet. A. 2009;149A:2088–92.

14. Obermann-Borst SA, Isaacs A, Younes Z, van Schaik RHN, van der Heiden IP, van Duyn CM, et al. General maternal medication use, folic acid, the MDR1 C3435T polymorphism, and the risk of a child with a congenital heart defect. Am. J. Obstet. Gynecol. 2011;204:236.e1-8.

15. Visser ST, Schuiling-Veninga CC, Bos JH, de Jong-van den Berg LTW, Postma MJ. The population-based prescription database IADB.nl : its development, usefulness in

outcomes research and challenges. Expert Rev. Pharmacoeconomics Outcome Res. 2013;13:285–92.

16. Schirm E, Meijer WM, Tobi H, De Jong-Van Den Berg LTW. Drug use by pregnant women and comparable non-pregnant women in the Netherlands with reference to the Australian classification system. Eur. J. Obstet. Gynecol. Reprod. Biol. 2004;114:182–8.

17. Bakker MK, Jentink J, Vroom F, Van Den Berg PB, De Walle HEK, De Jong-Van Den Berg LTW. Drug prescription patterns before, during and after pregnancy for chronic, occasional and pregnancy-related drugs in the Netherlands. BJOG. 2006;113:559–68.

18. Schirm E, Tobi H, de Jong-van den Berg LTW. Identifying parents in pharmacy data: a tool for the continuous monitoring of drug exposure to unborn children. J. Clin. Epidemiol. 2004;57:737–41.

19. Hertog F den, Verweij A, Mulder M, Sandersе C, Lucht F van der. Sociaaleconomische status: Wat is de huidige situatie? [Internet]. Volksgezond. Toekomst Verkenning, Natl. Kompas Volksgezond. versie 4.17. 2014. Available from: http://www.nationaalkompas.nl/bevolking/sociaaleconomische-status/wat-is-sociaaleconomische-status/#opleidingsniveau. Last accessed 23 March 2016.

20. O'Daniel J, Lucas J, Deverka P, Ermentrout D, Silvey G, Lobach DF, et al. Factors influencing uptake of pharmacogenetic testing in a diverse patient population. Public Health Genomics. 2009;13:48–54.

21. Zhang SC, Bruce C, Hayden M, Rieder MJ. Public perceptions of pharmacogenetics. Pediatrics. 2014;133:e1258–67.

22. Rogausch A, Prause D, Schallenberg A, Brockmöller J, Himmel W. Patients' and physicians' perspectives on pharmacogenetic testing. Pharmacogenomics. 2006;7:49–59.

23. Bevan JL, Lynch JA, Dubriwny TN, Harris TM, Achter PJ, Reeder AL, et al. Informed lay preferences for delivery of racially varied pharmacogenomics. Genet. Med. 2003;5:393–9.

24. Kobayashi E, Sakurada T, Ueda S, Satoh N. Public involvement in pharmacogenomics research: A national survey on patients' attitudes towards pharmacogenomics research and the willingness to donate DNA samples to a DNA bank in Japan. Cell Tissue Bank. 2011;12:71–80.

chapter 6

The risk of congenital heart anomalies following prenatal exposure to serotonin reuptake inhibitors

– Is pharmacogenetics the key?

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ABSTRACT

Serotonin reuptake inhibitors (SRIs) are often prescribed during pregnancy. Previous studies that found an increased risk of congenital anomalies, particularly congenital heart anomalies (CHA), with SRI use during pregnancy have created concern among pregnant women and healthcare professionals about the safety of these drugs. However, subsequent studies have reported conflicting results on the association between CHA and SRI use during pregnancy. These discrepancies in the risk estimates can potentially be explained by genetic differences among exposed individuals. In this review, we explore the potential pharmacogenetic predictors involved in the pharmacokinetics and mechanism of action of SRIs, and their relation to the risk of CHA. In general, the risk is dependent on the maternal concentration of SRIs and the fetal serotonin level/effect, which can be modulated by the alteration in the expression and/or function of the metabolic enzymes, transporter proteins and serotonin receptors involved in the serotonin signaling of the fetal heart development. Pharmacogenetics might be the key to understanding why some children exposed to SRIs develop a congenital heart anomaly and others do not.

INTRODUCTION

The use of antidepressants during pregnancy, particularly the use of selective serotonin reuptake inhibitors (SSRIs), has increased globally over the last few decades with the percentage of pregnant women users ranging between 1.2 to 6.2% up to 2005 (1–4). SSRIs were considered to cause fewer side effects compared to the first-generation of antidepressants until 2005, when a warning about the increased risk of fetal congenital heart anomalies (CHA) with SSRI use in pregnancy was released by the US Food and Drug Administration. This warning was shown to cause a decline, by 1.48 prescriptions per 1,000 women per month, in the prescribing of SSRIs among pregnant women in the US and Canada between 2005 and 2007 (5). Following this warning, many studies were carried out to evaluate the risk of congenital anomalies in children exposed to SSRIs during the first trimester of pregnancy. Most of these studies used data from healthcare monitoring systems and, while a large number of studies were done, the results have been inconsistent. Some studies reported an association, while other studies did not. With the emergence of genomic testing and personalized therapy, we now have the opportunity to explore the pharmacogenetic parameters that may explain why some children exposed to SSRIs develop a congenital heart anomaly and others do not.

This review presents our current knowledge about the associations between serotonin reuptake inhibitors (SRIs) and CHA, about the pharmacogenetic predictors that are potentially involved in the pharmacokinetics of SRIs during pregnancy and about the genetic predictors involved in the plausible biological mechanisms linking CHA to SRIs exposure, taking into consideration maternal and fetal factors. We use the classification of serotonin reuptake inhibitors (SRIs), because it includes the SSRIs and the serotonin/noradrenaline reuptake inhibitors (i.e., venlafaxine and duloxetine), both of which are based on the same mechanism of serotonin inhibition.

The risk of CHA associated with maternal use of SRIs during the first trimester of pregnancy

To have an insight in the current knowledge about the association between maternal use of SRIs during pregnancy and the risk of CHA, we performed a literature search for cohort and case-control studies published between January 2005 and May 2015 using the PubMed database (**Appendix 5.1**). Among 27 articles that were selected for review, no consistent pattern has been observed in the reported risk of CHA. A slight increase in risk was found, particularly for paroxetine, in a number of studies in various countries (see **Appendix 5.2**) (6–22), but a number of other studies reported no increased risk (see **Appendix 5.3**) (23–32). The dose of SRIs may also be an important determinant of the risk. A dose-effect relationship was observed

for paroxetine in one study (21), but it was not replicated in a subsequent study (33). The results of a meta-analysis by Wurst and colleagues in 2010 indicate an increased prevalence of cardiac malformations (OR 1.46, 95% CI 1.17-1.82) after paroxetine use during the first trimester of pregnancy (34). Another meta-analysis by Grigoriadis and colleagues in 2013, using adjusted data and excluding studies below a specified quality threshold, has also reported a significantly higher risk of cardiovascular malformations after maternal paroxetine use (RR=1.43, 95% CI 1.08-1.88) (35). Similar findings were also reported in a meta-analysis performed by Myles and colleagues in 2013 (OR 1.44, 95% CI 1.12-1.86) (36). The most recent meta-analysis, performed in 2015 including only prospective cohort studies, however, found no association of first trimester exposure to overall SRIs with an increased risk of CHA (37).

Most studies are population-based, linking drug exposure data from prescription databases with fetal outcome data from hospitals or birth defect registries. This approach has many limitations because these cohorts were not designed to investigate the fetal outcome following exposure to specific drugs (8,17,18,21,23–25,29,31). Consequently, many confounding factors cannot be addressed, and biases in exposure and outcome definitions have always been major considerations (38). While there are no perfect studies, each represents a different population and different risk factor assessments, and the study designs have improved over the years. A recent Bayesian analysis by National Birth Defects Prevention Study (NBDPS), based on the results of previous population-based studies and new NBDPS data, has reported that paroxetine and fluoxetine use during pregnancy were associated with a higher risk of several subtypes of CHA (39). Paroxetine was associated with atrial septal defects (ASDs) with posterior OR 1.8, 95% credible interval (CrI) 1.1-3.0 and right ventricular outflow tract obstruction defects (RVOTO) (posterior OR 2.4, 95% CrI 1.4-3.9). Fluoxetine was also associated with RVOTO (posterior OR 2.0, 95% CrI 1.4-3.1) and ventricular septal defects (VSDs) (posterior OR 1.4, 95% CrI 1.0-1.9). Although VSDs and ASDs are the most common subtypes of CHA (34% and 13%, respectively, of total CHA cases worldwide) (40), the absolute risk among children who were exposed to both SSRIs may still be considered low.

There are concerns among the patients who were taking these medications when they became pregnant, but there is still no definite answer if SRIs increase the risk of CHA in offspring. Because congenital heart anomalies are not common diseases (8/1 000 liveborns), and the number of cases exposed to SRIs is low, this inevitably leads to difficulties in obtaining a large enough sample to prove an association. Patients' worry about the risk may lead to noncompliance of SRIs among pregnant women, which may potentially cause serious consequences for their therapeutic management. The best practice at present is to assess the individual risk factors before prescribing SRIs

to pregnant women. Studies on the pharmacogenetics of SRIs can contribute to the understanding of the variability in risk estimates of SRI-induced CHA, and may assist in identifying mothers who are at a higher risk of having a child with CHA.

Pharmacogenetic predictors of SRI pharmacokinetics

During pregnancy, the pharmacokinetics (absorption, distribution, metabolism and excretion) of SRIs are known to be altered because of the physiological changes associated with pregnancy. These changes include increased total body water (including blood volume), reduced albumin concentration (by up to 10g/L and crucial for SRIs with high protein binding, e.g. fluoxetine, sertraline, paroxetine, duloxetine), modulation of metabolic enzymes by pregnancy hormones and increased renal function and drug clearance (41,42). These physiological adaptations influence the level of SRIs in the maternal circulation, and subsequently affect the amount transferred to the fetus (**Figure 1**).

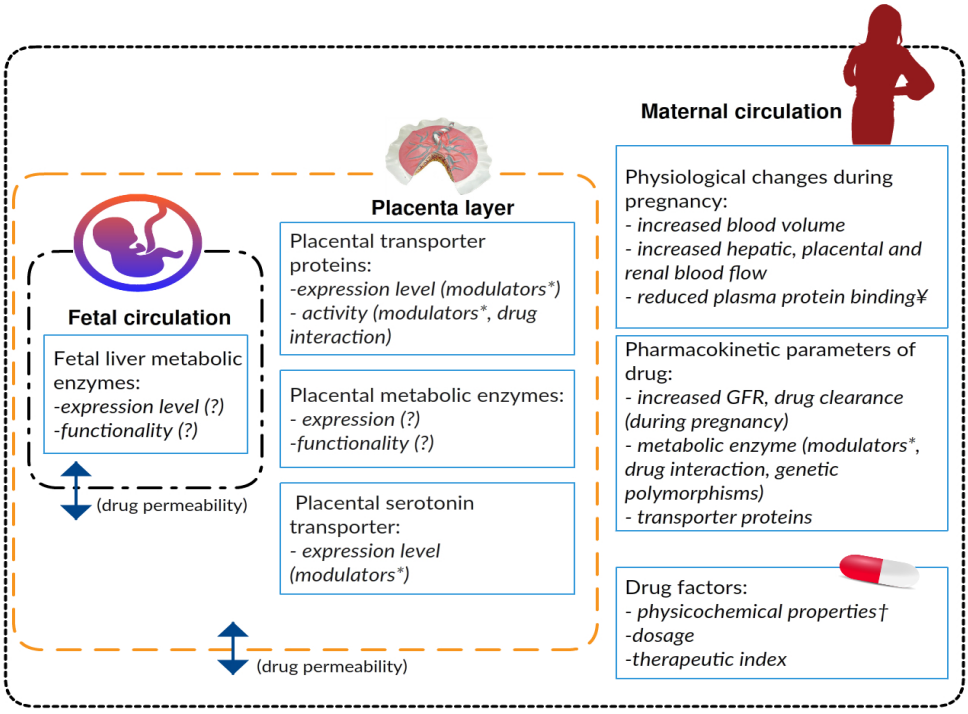

Figure 1: Factors influencing fetal drug exposure divided into factors in the maternal circulation, placenta layer and fetal circulation: *, substances that induce or inhibit the activity of enzymes/transporters, which may include pregnancy hormones and other drugs taken by the mother; ¥, important for serotonin reuptake inhibitors (SRIs) with high protein binding (e.g., fluoxetine, paroxetine, sertraline); †, molecular size, polarity, charge, lipophilicity of the drug; GFR, glomerular filtration rate; the double headed arrows indicate passive diffusion of drugs.

The passage and metabolism of SRIs supposedly occur through the yolk sac in the early stage of the first trimester up until the placenta forms in the late stage of the first trimester. Unlike other species, little is known about the transporters and binding proteins in the human yolk sac relevant for the availability and toxicity of chemicals to the embryo (43,44). Nevertheless, drug transport in early pregnancy is postulated to be affected by pH gradients and protein binding between maternal and fetal compartments (44).

SRIs, with molecular weights around 300 g/mol, are able to cross the placenta, although the amount transferred in the first trimester is difficult to measure. In term placenta, the mean ratio of umbilical cord concentration to maternal serum concentration varies among SRIs depending on their molecular weight and polarity. The highest ratio was found for venlafaxine (range 0.72-1.1) and citalopram (0.71-0.83), followed by fluoxetine (0.64-0.73). The transfer of paroxetine and sertraline across the placenta seemed to be much lower (0.15-0.54 and 0.29-0.33, respectively) (45–47). However, term data may not be representative of the first trimester of pregnancy.

Maternal metabolic enzymes

The most important enzymes in SRI metabolism are the cytochrome P450 (CYP) enzymes, including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 isoenzymes. These enzymes are responsible for the inactivation of SRIs, and are mainly expressed in the maternal liver, with the exception of CYP3A4, which is also expressed in the small intestine (48). In the placenta, mRNAs were found for CYP1A2, CYP2D6, CYP3A4, CYP3A5 and CYP3A7 in the first trimester, but their protein expression and functionality was not widely characterized. Meanwhile, for CYP1A1, the mRNA, protein and functional activity were detected during the first trimester, but not in subsequent trimesters (49,50). In term placenta samples, high expression and functional activity were detected for CYP19A1, which is responsible for the conversion of androgens to oestrogens (51).

The metabolism of each SRI agent varies depending on its affinity towards the isoenzymes. Fluoxetine, paroxetine, venlafaxine and duloxetine are metabolized to a major extent by CYP2D6, and to a lesser extent by CYP1A2 (for duloxetine), CYP2C9 and CYP3A4 (for fluoxetine), and CYP2C19 and CYP3A4 (for venlafaxine) (52–55). CYP2C19 is the major metabolic enzyme for citalopram and escitalopram (CYP3A4 and CYP2D6 to a lesser extent); CYP3A4 for sertraline (CYP2B6, CYP2C9, CYP2C19, CYP2D6 to a lesser extent); and CYP2D6, CYP1A2 and CYP3A4 for fluvoxamine (52,53,56). Unlike other SRIs, fluoxetine is a prodrug that will be

metabolized to an active enantiomer, norfluoxetine, to promote pharmacological action. As for CYP19A1, there is no data found for the metabolism of SRIs with this enzyme.

The knowledge of genetic variation of CYP enzymes has been used in practice for dose modification of certain drugs (57–59). The polymorphisms of *CYP2C9*, *CYP2C19* and *CYP2D6* are well documented and cause changes in protein expression and function, leading to alterations in the plasma level of substrate drugs that consequently affect the clinical efficacy and toxicity (**Table 1**). A dosing guideline for SSRIs (paroxetine, fluvoxamine, citalopram, escitalopram and sertraline) for *CYP2D6* and *CYP2C19* genotypes was recently introduced (59) based on the results of numerous clinical and association studies (48,60). Our great concern is for mothers with single nucleotide polymorphisms (SNP) leading to a poor metabolizer phenotype (i.e. *CYP2D6*3/*4*, **4/*4*, **5/*5*, **5/*6* or *CYP2C19*2/*2*, **2/*3*, **3/ *3*), who are at a greater risk of SRI overdosing and side effects. The slower metabolism of SRIs leads to a greater concentration of these drugs in the mother's bloodstream, which could lead to a higher concentration crossing the placental barrier.

Only few studies have focused on the effect of CYP enzyme genotypes on the SRI pharmacokinetics during pregnancy. The maternal *CYP2D6* genotype of intermediate and poor metabolizers showed an increase in plasma concentration of paroxetine of 0.82 mg/L (95% CI 0.42-1.22) for each week over the course of pregnancy, which is in contrast to the decline observed among extensive and ultra-rapid metabolizers (61). *CYP2C9*2* and *CYP2C9*3* were associated with a lower activity of CYP2C9 enzymes, which are thought to be responsible for the metabolism of fluoxetine, sertraline and venlafaxine, but these studies used minimal data and found a low strength of association (62,63). Furthermore, the effect of genetic polymorphisms of *CYP1A2* has been studied less and is thought to contribute little to the pharmacokinetics of SRIs (60).

Apart from genetic polymorphisms, the inhibition or induction of CYP enzymes by certain drugs taken together with SRIs will also affect the metabolism of SRIs. For example co-medication with a CYP2D6 inhibitor was shown to be associated with increased plasma concentrations of citalopram, sertraline and venlafaxine, similar to the effect of the poor metabolizer phenotype (64).

Fetal metabolic enzymes

Little is known about the expression or activity of metabolic enzymes in the fetus. In the fetal liver, CYP3A7 has previously been reported as the dominant isoenzyme, and its expression decreases postnatally when it is substituted by CYP3A4 (65). Genetic

polymorphisms of *CYP3A4* contribute to a minor extent to drug pharmacokinetics and clinical therapy, including that of SRIs (66). However, more recent evidence suggests high phenotypic interindividual variability in fetal expression of CYP3A4 and CYP3A7, and that gestational age is not the most important covariate (67). Fetal SNP *CYP3A7*1E* has been clinically demonstrated to reduce the efficacy of betamethasone in stimulating fetal lung maturity following maternal antenatal administration, although the exact mechanism remains unknown (68). Meanwhile, in adult liver and intestinal cells, the interindividual variability in CYP3A7 expression was very pronounced, while the variant alleles of *CYP3A7*1B* and *CYP3A7*1C* were found to be associated with an increase in enzyme expression (65). However, with regard to the metabolism of SRIs, there is no data so far indicating the role of CYP3A7 in the metabolism of these drugs. Although CYP2C9 and CYP2C19 were also shown to have functional activity in some fetal liver samples, there is a high variability in the expression profile between samples (69,70). Among 60 fetuses aged less than 30 weeks of gestational age, CYP2D6 protein expression (5% as of adult) and functional activity (1% as of adult) was detected in only 30 of all liver samples (71).

Overall, the expression and activity of CYP2D6 in the first and second trimester fetal samples were either undetectable or very low, and the expression and activity increased in the third trimester (72). In general, our knowledge of fetal metabolic enzymes is limited, and a high interindividual variability in the expression profile was observed. As the activity of these enzymes in the fetal liver may need further investigations, the contribution of these enzymes to the fetal metabolism of SRIs, particularly in the first trimester, is probably minor.

Placental transporter proteins

The placenta expresses several transporter proteins that are involved in the regulation of the chemical environment of the fetus by transporting and removing toxic substrates (73–75). Meanwhile, transporter proteins expressed in other organ cells, e.g. the intestine, kidney and liver, are important for the absorption, distribution and excretion of SRIs and their metabolites. One of the most-studied placental transporters is P-glycoprotein (P-gp), which is expressed in the maternal-facing membrane of the placental syncytiotrophoblast (76,77). P-gp facilitates the efflux transport of a wide range of substrate drugs, including SRIs (78–81). The expression of P-gp is highest in the early stages of pregnancy (82,83) denoting the role of P-gp in limiting the fetal exposure to xenobiotics or other harmful substances. Our previous study has shown that the inhibition of P-gp efflux activity of drug substrates was associated with an increased risk of congenital anomalies for drugs that were

associated with certain types of congenital anomalies (84).

The polymorphisms of the *ABCB1* gene encoding for P-gp have been studied extensively with a focus on its effect on the pharmacokinetics, clinical efficacy and toxicity of antidepressants (85–88). These studies focused on P-gp expression in the blood brain barrier, which plays an important role in the bioavailability of these antidepressants in the brain. Under normal conditions, P-gp effluxes the substrates out of the brain cells, which can either lead to lower efficacy or reduced side effects of the substrates. Several *ABCB1* SNPs (*3435C>T*, *1236C>T*, *2677G>T*) previously associated with reduced P-gp expression, have also been associated with increased efficacy or increased side effects that lead to switching and discontinuation of therapy (89–91). In the placenta, *3435C>T*, *1236C>T* and *2677G>T* SNPs were associated with a reduced mRNA and/or protein expression of P-gp in human placental samples, suggesting a weaker fetal protection against potential teratogens (92–94). This finding was supported by two clinical studies that found an increased risk of cleft lip (95) and CHA (96) associated with a maternal *3435T* variant allele in mothers taking any medication during the first trimester of pregnancy. The risk was even higher in mothers who did not take folic acid supplements (95,96). Several other *ABCB1* SNPs relevant to the pharmacogenomics of SRIs were found to be associated with SRI response and adverse events. In **Table 1**, the predicted effect on protein expression/activity in the placenta and the predicted effect on fetal exposure to SRIs are shown.

Maternal metabolic CYP enzymes and placental transporters both play an important role in determining the fetal SRI exposure. Metabolic enzymes affect the concentration of SRIs in the maternal circulation, while placental P-gp determines the amount transported into the fetal circulation. Any changes in the expression and function of these enzymes and transporters may lead to variation in fetal SRI exposure. Despite the need to evaluate the extent of fetal SRI exposure, there are a limited number of ways to measure it directly, e.g. using animal studies and *in vivo*, *in vitro* or *ex vivo* placental transfer models (74,75,97). When examining the genetic factors, one should take both the mother and the fetus into consideration as both provide several mechanisms to limit fetal exposure to SRIs.

Table 1: Overview of polymorphisms significantly associated with SRI pharmacokinetics and their predicted effect on fetal SRI exposure

Gene	SNPs	rs Numbers	MAF (%)[a]			Pharmacokinetics and/or clinical effects	Phenotype (predicted expression/activity)	Predicted effect on fetal SRI exposure[b]	SRIs likely to be affected
			Caucasians	Asians	Africans				
CYP1A2	*-3113G>A*	rs2069521	3	8	11	Increased severity of side effects of escitalopram (98)	Increased [c]	Reduced	Fluvoxamine, duloxetine
	-10+103 T>G	rs2069526	3	8	12	Increased severity of side effects of escitalopram (98)	Increased [c]		
	832-249 C>T	rs4646425	3	8	0	Increased severity of side effects of escitalopram (98), reduced efficacy of paroxetine (99)	Increased [c]		
	1253+81 T>C	rs4646427	3	8	11	Increased severity of side effects of escitalopram (99)	Increased [c]		
	1042+43 G>A	rs2472304	59	16	4	Increased efficacy of paroxetine (99)	Reduced [c]	Increased	
	1548C>T	rs2470890	59	16	3	Increased efficacy of paroxetine (99)	Reduced [c]		
CYP2C9	**2*	rs1799853	11	0	4	Reduced metabolism of fluoxetine (62,63)	Reduced [d]	Increased	Fluoxetine, sertraline, venlafaxine
	**3*	rs1057910	7	3	2	Reduced metabolism of fluoxetine (62,63)	Reduced [d]	Increased	
CYP2C19	**2*	rs4244285	15	33	17	Reduced tolerance to citalopram [100] and reduced metabolism of escitalopram (101)	Reduced [c, d]	Increased	Citalopram*, escitalopram*, sertraline, venlafaxine
	**3*	rs4986893	0	5	0	Reduced metabolism of escitalopram (102)	Reduced [d]	Increased	

CYP2C19	**17*	rs12248560	23	2	22	Increased metabolism of citalopram (103), escitalopram (102,104)	Increased [d]	Reduced	Citalopram*, escitalopram*, sertraline, venlafaxine
CYP2D6	**3*	rs35742686	2	0	0	Reduced metabolism of escitalopram (104), venlafaxine (105)	No activity [d]	Increased	Paroxetine*, fluoxetine*, venlafaxine*, fluvoxamine, sertraline
	**4*	rs3892097	19	0	6	Reduced metabolism of escitalopram (104), venlafaxine (105,106)	No activity [d]	Increased	
	**5*	whole gene deletion	4	7.2	ND	Reduced metabolism of paroxetine (107)	No activity [d]	Increased	
	**10*	rs1065852	20	52	9	Reduced metabolism of paroxetine (107)	Reduced [d]	Increased	
ABCB1 (P-gp)	*3435C>T*	rs1045642	53	40	15	Increased efficacy of escitalopram (108,109), venlafaxine (109), increased concentration of fluvoxamine (110), a group of antidepressants (89)	Reduced [c,d]	Increased	Paroxetine, fluoxetine, venlafaxine, fluvoxamine, sertraline, venlafaxine, citalopram, escitalopram
	1236C>T	rs1128503	43	66	14	Increased concentration and side effects of antidepressants (89)	Reduced [c,d]	Increased	
	3489+1573G>A	rs1882478	26	57	63	Increased efficacy of escitalopram (108)	Reduced [c]	Increased	

Abbreviations: MAF, minor allele frequency; ND: no data; CYP, cytochrome P450; P-gp, P-glycoprotein; SSRIs, selective serotonin reuptake inhibitors. *Causes dose modification in patients with polymorphic variants (57-59); [a] MAFs from SNPedia, www.cypalleles.ki.se, PharmGkb, 1000 Genomes, HapMap; [b] predicted effect on fetal SRI exposure: the exposure is predicted to be increased if the expression/activity of CYP enzymes is reduced, leading to an increase in SRI concentration in the maternal circulation and more SRI transported through the placenta (and vice versa); [c] based on clinical data [d] based on pharmacokinetic data.

Table 1 (cont.)

Gene	SNPs	rs nmbers	MAF (%)[a]			Pharmacokinetics and/or clinical effects	Phenotype (predicted expression/activity)	Predicted effect on fetal SRI exposure[b]	SRIs likely to be affected
			Caucasians	Asians	Africans				
ABCB1 (P-gp)	*2677G>T*	rs2032582	43	45	3	Reduced concentration and efficacy of citalopram (111), increased efficacy of paroxetine (90)	Increase or reduced [c, d]	Increased or reduced	Paroxetine, fluoxetine, venlafaxine, fluvoxamine, sertraline, venlafaxine, citalopram, escitalopram
	2493+49T>C	rs2035283	13	6	22	Increased efficacy of paroxetine (112) and side effects of SSRIs (113)	Reduced [c]	Increased	
	2481+24G>A	rs2235040	13	6	20	Increased efficacy of paroxetine (112) and side effects of SSRIs (113)	Reduced [c]	Increased	
	2482-236A>G	rs4148739	13	6	22	Increased efficacy of SSRIs (114)	Reduced [c]	Increased	
	61A>G	rs9282564	9	0	0	Increased efficacy of paroxetine (115)	Reduced [c]	Increased	
	287-1234G>C	rs10256836	29	15	8	Reduced efficacy of escitalopram (108)	Increased [c]	Reduced	
	2927+314G>A	rs28401781	13	6	20	Increased efficacy of SSRIs (114)	Reduced [c]	Increased	

Abbreviations: MAF, minor allele frequency; ND: no data; CYP, cytochrome P450; P-gp, P-glycoprotein; SSRIs, selective serotonin reuptake inhibitors. *Causes dose modification in patients with polymorphic variants (57-59); [a] MAFs from SNPedia, www.cypalleles.ki.se, PharmGkb, 1000 Genomes, HapMap; [b] predicted effect on fetal SRI exposure: the exposure is predicted to be increased if the expression/activity of CYP enzymes is reduced, leading to an increase in SRI concentration in the maternal circulation and more SRI transported through the placenta (and vice versa); [c] based on clinical data [d] based on pharmacokinetic data.

Pharmacogenetic predictors of CHA associated with exposure to SRIs

Serotonin (5-HT) is a neurotransmitter that also acts as a growth factor and is an important regulatory factor during a critical period of embryo development. The period of about 20–70 days following fertilization involves the formation of the brain (43,116). The fetal heart also undergoes gross morphological changes within the first 112 days of development, including septation (between 35 and 53 days), formation of the valve components (between 49 and 56 days) and delamination of the leaflets into the tricuspid valve (between 56 and 112 days) (117). The cardiac morphogenesis is dependent on the migration, survival and proliferation of neural crest cells, which are regulated by 5-HT, mainly via the 5-HT2B receptor (118-119). 5-HT is also one of the factors in the signaling cascade driving the establishment of laterality in heart cells. Disruptions in the laterality cascade result in laterality defects of the heart such as atrial isomerism, transposition of the great arteries, double outlet right ventricle and common truncus arteriosus (120). The pathology of heart defects has also been postulated to be associated with the pattern of intracardiac blood flow (121), which is another link between 5-HT and heart development because 5-HT acts as a potent vasoconstrictor and is important in maintaining an optimal uteroplacental blood flow (122).

During embryogenesis, the embryo is supplied with 5-HT from the maternal blood. 5-HT in the maternal circulation can be transported to the fetal circulation by the serotonin transporter (SERT) expressed in the placenta, and signals through serotonin receptors in the fetus (123). However, in depressed mothers, there is an abnormally reduced function of the serotonergic system in the brain. It is commonly agreed that for women who took antidepressants during pregnancy, the effect on fetal outcome is difficult to measure and disentangle from the effect of depression itself, since there is a lack of evidence to conclude whether depression itself poses an increased risk for CHA (8,124,125). Therefore, we are looking for other possible factors, for instance the polymorphisms of SERT and fetal serotonin receptors that might possibly be among the predictors of the risk of CHA.

Serotonin transporter in fetal cardiac cells and in the placenta

Based on animal and *in vitro* studies, the effect of SRIs on embryonic heart development can occur via modulation of serotonin transporter levels and prenatal 5-HT levels (126,127). In humans, this effect occurs via direct exposure to SRIs, which are readily passed through the placenta, to the fetal serotonergic system. In the fetus, SRIs inhibit SERT expressed in the fetal cardiac cells, which subsequently reduce the transport of 5-HT into the cells and could, in theory, disturb the normal development

of the heart. In addition, SRIs can also inhibit SERT expressed in the placenta, which will limit the transport of 5-HT and/or other important growth factors through the placenta for fetal use (116).

Polymorphisms of the *SLC6A4* gene encoding for SERT may also play a role in the serotonin signaling in fetal heart development. The genetic variation in the SERT promoter gene region, SERTLPR (formerly 5-HTTLPR), was previously associated with SRI response and adverse risk events **(Table 2)**. This insertion/deletion polymorphism includes a short (S) and a long (L) allele, and the S allele is associated with reduced activity in placental tissue and increased risk of adverse neonatal outcome events associated with SRI use (128,129). Another polymorphism, rs25531 is putatively located in the sixth repeat of the SERTLPR, with L_A or L_G alleles. The expression of SERT is known to be higher in the L_A allele, while it is reduced in the L_G allele to a level similar to the SERTLPR S allele (130). Since the SRIs inhibit SERT, less expression of this transporter may increase the inhibition rate. That is, fetuses with S or L_G genotype are likely to receive a higher "effective" dose, considering there is less SERT to be blocked. As a consequence, a lower amount of 5-HT is permitted into the fetal circulation (128) to regulate normal cardiac morphogenesis.

Fetal serotonin receptors

5-HT activates seven distinct families of 5-HT receptors with 16 subtypes, and most of the receptors are G-protein coupled (131). Several SNPs of genes encoding for 5-HT1A, 1B, 2A and 3B were reported to be correlated with SRI response and side effects, which might be related to the alteration in receptor expression or activity in the nervous system. Some polymorphisms were associated with a better response to SRIs, for example, of the *HTR2A* rs6314*,* rs1928040*,* rs7997012*,* rs6311 (132-137), and of the *HTR1A* rs1364043 and of the *HTR1B* rs6296 in the treatment of citalopram (138). Other polymorphisms, on the other hand, were shown to reduce the response of several SRIs, e.g. *HTR1A* rs6295 in the treatment of fluoxetine, fluvoxamine, and citalopram (138-141). Furthermore, an increase in side effects of paroxetine was reported among patients with *HTR2A* rs6313, *HTR3B* rs1176744 and *HTR3B* rs3831455 (132,142,143). Unlike other genes, there are limited data on the polymorphisms of the gene encoding for the 5-HT2B receptor, which is more important, in this regard, in the developmental stage of the fetal heart (118,119,144).

When a woman in the first trimester of pregnancy is required to take SRIs, we can assume a reduced amount of 5-HT may be transferred into the fetal circulation following the inhibition of placental SERT. The reduced concentration of 5-HT in the fetal circulation, together with the changes in the expression and/or activity of the 5-HT receptors, may subsequently affect the normal development of the fetal heart.

Table 2: Polymorphisms of the serotonin transporter (SERT) and their predicted effect on CHA risk in offspring exposed *in utero*

Gene	SNPs	rs Numbers	MAF (%) [a]			Clinical effects	Phenotype (predicted enzyme/ protein expression or activity)	Predicted effect on CHA risk [b]	SRIs likely to be affected
			Caucasians	Asians	Africans				
SLC6A4 (SERT)	*SERTLPR* or *5-HTTLPR* (S and L alleles)	rs4795541	40 (S)	80 (S)	17 (S)	S-allele: poor response to venlafaxine (147), fluoxetine (139,148), increase side effects of fluvoxamine (137), citalopram (149), escitalopram (150), paroxetine (151) and overall SSRIs (152,153)	Reduced with S allele	Increased	Fluoxetine, citalopram, sertraline, paroxetine, escitalopram, fluvoxamine
	-1936A>G (*SERTLPR* L_A/ L_G allele)	rs25531	9	8	21	L_G allele: increased risk of side effects and poor response citalopram (149) and overall SSRIs (153)	Reduced with L_G allele	Increased	
	5HTT VNTR (9,10 or 12 repeat)	rs57098334	47 (10)	10 (10)	26 (10)	12 allele was associated with higher rates of side effects of SSRIs (153)	Increased transcription with 12 repeats	Reduced	

Abbreviations: MAF, minor allele frequency; S, short allele; L, long allele. [a] MAFs from SNPedia, www.cypalleles.ki.se, PharmGkb, 1000 Genomes, HapMap; [b] predicted effect on CHA risk, based on hypothetical conditions (see text).

Other genes

Most CHA have a complex aetiology, with some caused by a Mendelian trait or a chromosomal aberration. The genetic aetiology of CHA is not yet well understood, and the known genetic causes of CHA account for less than 20% of CHA cases (121,145). The genetic variations of other genes involved in the pathway of fetal heart development are not emphasized in this review, but should also be taken into consideration in determining the true causal relationship between SRI exposure and CHA. A recent study found that the placenta of SSRI-treated mothers had a lower expression of the ROCK2 gene, which is thought to play a role in the development of the cardiovascular system of the fetus, as compared to untreated depressed and healthy mothers (125). In contrast, a study with a similar setting, but focused on the neurotrophic growth factor signaling pathway, found an increased level of the ROCK2 gene and of phosphorylated ROCK2 in SSRI-treated women in comparison to depressed and healthy women (146). Despite the contrary findings, both studies speculated that ROCK2 expression could be altered in the placenta of SSRI-treated women, and might disturb the normal development of the fetal cardiovascular system. Another aspect to be considered is the effect of fetal epigenetic programming, which is currently being investigated as the candidate molecular mechanism underlying physiological alterations in exposed fetuses (125).

Our understanding of the biological plausibility, corroborated by the evidence, may indicate that prenatal use of SRIs causes an alteration in the signaling pathway important for the development of the fetal heart. This alteration is also dependent on the pharmacokinetics of SRIs in maternal and fetal circulation. Moreover, any alteration in the expression and/or function of the enzymes, proteins, transporters and receptors involved in the signaling, modulated by the genetic polymorphisms, may theoretically alter the risk of CHA.

CONCLUSION

The scope of research on the risk of CHA associated with prenatal exposure to SRIs should be extended to include the role of pharmacogenetics in pregnancy. While implementing the results in clinical practice may still seem a distant prospect, we need to begin developing theories and doing model simulations that will help us understand the complex interactions between maternal and fetal genetics and their effect on fetal SRI exposure and the risk of CHA. A better understanding of these interactions is a crucial step toward considering personalized drug treatment models for pregnant women with depression.

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REFERENCES

1. Alwan S, Reefhuis J, Rasmussen SA, Friedman JM: Patterns of antidepressant medication use among pregnant women in a United States population. J Clin Pharmacol 2011;51:264–270.

2. Bakker MK, Kölling P, van den Berg PB, de Walle HEK, de Jong van den Berg LTW: Increase in use of selective serotonin reuptake inhibitors in pregnancy during the last decade, a population-based cohort study from the Netherlands. Br J Clin Pharmacol 2008 May;65:600–6.

3. Charlton R, Jordan S, Pierini A, Garne E, Neville A, Hansen A, et al.: Selective serotonin reuptake inhibitor prescribing before, during and after pregnancy: a population-based study in six European regions. BJOG 2014 Oct 28;doi: 10.1111/1471-0528.13143.

4. Andrade SE, Raebel MA, Brown J, Lane K, Livingston J, Boudreau D, et al.: Use of antidepressant medications during pregnancy: a multisite study. Am J Obstet Gynecol 2008;198:194–195.

5. Bobo W V., Epstein Jr. RA, Hayes RM, Shelton RC, Hartert T V., Mitchel E, et al.: The effect of regulatory advisories on maternal antidepressant prescribing, 1995-2007: An interrupted time series study of 228,876 pregnancies. Arch Womens Ment Health 2014;17:17–26.

6. Bérard A, Zhao J-P, Sheehy O: Sertraline use during pregnancy and the risk of major malformations. Am J Obstet Gynecol 2015;212:e1-12.

7. Wemakor A, Casson K, Garne E, Bakker M, Addor M-C, Arriola L, et al.: Selective serotonin reuptake inhibitor antidepressant use in first trimester pregnancy and risk of specific congenital anomalies: a European register-based study. Eur J Epidemiol 2015;(DOI)10.1007/s10654-015-0065-y.

8. Ban L, Gibson J, West J, Fiaschi L, Sokal R, Smeeth L, et al.: Maternal depression, antidepressant prescriptions, and congenital anomaly risk in offspring: A population-based cohort study. BJOG An Int J Obstet Gynaecol 2014;121:1471–1481.

9. Knudsen T, Hansen A, Garne E, Andersen A-M: Increased risk of severe congenital heart defects in offspring exposed to selective serotonin-reuptake inhibitors in early pregnancy – an epidemiological study using validated EUROCAT data. BMC Pregnancy Childbirth 2014;14:333.

10. Polen KN, Rasmussen SA, Riehle-Colarusso T, Reefhuis J, Study NBDP: Association between reported venlafaxine use in early pregnancy and birth defects, national birth defects prevention study, 1997-2007. Birth defects Res A, Clin Mol Teratol 2013 Jan;97:28–35.

11. Malm H, Artama M, Gissler M, Ritvanen A: Selective serotonin reuptake inhibitors and risk for major congenital anomalies. Obstet Gynecol 2011 Jul;118:111–20.

12. Reis M, Kallen B: Delivery outcome after maternal use of antidepressant drugs in pregnancy: an update using Swedish data. Psychol Med 2010;40:1723–1733.

13. Bakker MK, Kerstjens-Frederikse WS, Buys CH, de Walle HE, de Jong-van den Berg LT: First-trimester use of paroxetine and congenital heart defects: a population-based case-control study. Birth defects Res A, Clin Mol Teratol 2010 Feb;88:94–100.

14. Merlob P, Birk E, Sirota L, Linder N, Berant M, Stahl B, et al.: Are selective serotonin reuptake inhibitors cardiac teratogens? Echocardiographic screening of newborns with persistent heart murmur. Birth defects Res A, Clin Mol Teratol 2009;85:837–841.

15. Pedersen LH, Henriksen TB, Vestergaard M, Olsen J, Bech BH: Selective serotonin reuptake inhibitors in pregnancy and congenital malformations: population based cohort study. BMJ 2009 Sep;339:b3569.

16. Diav-Citrin O, Shechtman S, Weinbaum D, Wajnberg R, Avgil M, Gianantonio ED, et al.: Paroxetine and fluoxetine in pregnancy: a prospective, multicentre, controlled, observational study. Br J Clin Pharmacol 2008 Nov;66:695–705.

17. Oberlander TF, Warburton W, Misri S, Riggs W, Aghajanian J, Hertzman C: Major congenital malformations following prenatal exposure to serotonin reuptake inhibitors and benzodiazepines using population-based health data. Birth defects Res B, Dev Reprod Toxicol 2008 Feb;83:68–76.

18. Cole JA, Ephross SA, Cosmatos IS, Walker AM: Paroxetine in the first trimester and the prevalence of congenital anomalies. Pharmacoepidemiol Drug Saf 2007;16:1075–1085.

19. Louik C, Lin AE, Werler MM, Hernandez-Diaz S, Mitchell AA: First-trimester use of selective serotonin-reuptake inhibitors and the risk of birth defects. N Engl J Med 2007 Jun;356:2675–2683.

20. Källén BAJ, Olausson PO: Maternal use of selective serotonin re-uptake inhibitors in early pregnancy and infant congenital malformations. Birth Defects Res Part A - Clin Mol Teratol 2007;79:301–308.

21. Berard A, Ramos E, Rey E, Blais L, St-Andre M, Oraichi D: First trimester exposure to paroxetine and risk of cardiac malformations in infants: the importance of dosage. Birth defects Res B, Dev Reprod Toxicol 2007 Feb;80:18–27.

22. Wogelius P, Nørgaard M, Gislum M, Pedersen L, Munk E, Mortensen PB, et al.: Maternal use of selective serotonin reuptake inhibitors and risk of congenital malformations. Epidemiology 2006;17:701–704.

23. Furu K, Kieler H, Haglund B, Engeland A, Selmer R, Stephansson O, et al.: Selective serotonin reuptake inhibitors and venlafaxine in early pregnancy and risk of birth defects: population based cohort study and sibling design. BMJ Open 2015;350:h1798–h1798.

24. Huybrechts KF, Palmsten K, Avorn J, Cohen LS, Holmes LB, Franklin JM, et al.: Antidepressant use in pregnancy and the risk of cardiac defects. N Engl J Med 2014;370:2397–2407.

25. Vasilakis-Scaramozza C, Aschengrau A, Cabral H, Jick SS: Antidepressant use during early pregnancy and the risk of congenital anomalies. Pharmacotherapy 2013;33:693–700.

26. Margulis A V, Abou-Ali A, Strazzeri MM, Ding Y, Kuyateh F, Frimpong EY, et al.: Use of selective serotonin reuptake inhibitors in pregnancy and cardiac malformations: a propensity-score matched cohort in CPRD. Pharmacoepidemiol Drug Saf 2013;22:942–951.

27. Klieger-Grossmann C, Weitzner B, Panchaud A, Pistelli A, Einarson T, Koren G, et al.: Pregnancy Outcomes Following Use of Escitalopram: A Prospective Comparative Cohort Study. J Clin Pharmacol 2012;52:766–770.

28. Einarson A, Choi J, Einarson TR, Koren

G: Incidence of major malformations in infants following antidepressant exposure in pregnancy: Results of a large prospective cohort study. Can J Psychiatry 2009;54:242–246.

29. Wichman CL, Moore KM, Lang TR, St Sauver JL, Heise RHJ, Watson WJ: Congenital heart disease associated with selective serotonin reuptake inhibitor use during pregnancy. Mayo Clin Proc 2009;84:23–27.

30. Lennestål R, Källén B: Delivery outcome in relation to maternal use of some recently introduced antidepressants. J Clin Psychopharmacol 2007;27:607–613.

31. Davis RL, Rubanowice D, McPhillips H, Rabael MA, Andrade SE, Smith D, et al.: Risk of congenital malformations and perinatal events among infants exposed to antidepressant medications during pregnancy. Pharmacoepidemiol Drug Saf 2007;16:1086–1094.

32. Alwan S, Reefhuis J, Rasmussen SA, Olney RS, Friedman JM, Study NBDP: Use of selective serotonin-reuptake inhibitors in pregnancy and the risk of birth defects. N Engl J Med 2007 Jun;356:2684–2692.

33. Jimenez-Solem E, Andersen JT, Petersen M, Broedbaek K, Jensen JK, Afzal S, et al.: Exposure to selective serotonin reuptake inhibitors and the risk of congenital malformations: a nationwide cohort study. BMJ Open 2012 Jan;2:e001148.

34. Wurst KE, Poole C, Ephross SA, Olshan AF: First trimester paroxetine use and the prevalence of congenital, specifically cardiac, defects: a meta-analysis of epidemiological studies. Birth defects Res A, Clin Mol Teratol 2010;88:159–170.

35. Grigoriadis S, VonderPorten EH, Mamisashvili L, Roerecke M, Rehm J, Dennis C-L, et al.: Antidepressant exposure during pregnancy and congenital malformations: is there an association? A systematic review and meta-analysis of the best evidence. J Clin Psychiatry 2013 Apr;74:e293-308.

36. Myles N, Newall H, Ward H, Large M: Systematic meta-analysis of individual selective serotonin reuptake inhibitor medications and congenital malformations. Aust N Z J Psychiatry 2013;47:1002–12.

37. Wang S, Yang L, Wang L, Gao L, Xu B, Xiong Y: Selective Serotonin Reuptake Inhibitors (SSRIs) and the Risk of Congenital Heart Defects: A Meta-Analysis of Prospective Cohort Studies. J Am Heart Assoc 2015;4:e001681.

38. Grzeskowiak LE, Gilbert AL, Morrison JL: Exposed or not exposed? Exploring exposure classification in studies using administrative data to investigate outcomes following medication use during pregnancy. Eur J Clin Pharmacol 2012;68:459–467.

39. Reefhuis J, Devine O, Friedman JM, Louik C, Honein MA: Specific SSRIs and birth defects: bayesian analysis to interpret new data in the context of previous reports. BMJ 2015;350:h3190.

40. Van Der Linde D, Konings EEM, Slager MA, Witsenburg M, Helbing WA, Takkenberg JJM, et al.: Birth prevalence of congenital heart disease worldwide: A systematic review and meta-analysis. J Am Coll Cardiol 2011;58:2241–2247.

41. Isoherranen N, Thummel KE: Drug metabolism and transport during pregnancy : How does drug disposition change during pregnancy and what are the mechanisms that cause such changes ? Drug Metab Dispos 2013;41:256–262.

42. Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H: Anatomical, physiological and metabolic changes with gestational age during normal pregnancy. Clin Pharmacokinet 2012;51:365–396.

43. Peters P, Miller RK, Schaefer C: General

commentary on drug therapy and drug risks in pregnancy; in Schaefer C, Peters P, Miller RK (eds): Drugs during pregnancy and lactation, ed 3. Munich, Elsevier B.V., 2014, pp 1–23.

44. Carney EW, Scialli AR, Watson RE, DeSesso JM: Mechanisms regulating toxicant disposition to the embryo during early pregnancy: An interspecies comparison. Birth Defects Res Part C - Embryo Today Rev 2004;72:345–360.

45. Hendrick V, Stowe ZN, Altshuler LL, Hwang S, Lee E, Haynes D: Placental passage of antidepressant medications. Am J Psychiatry 2003;160:993–996.

46. Rampono J, Proud S, Hackett LP, Kristensen JH, Ilett KF: A pilot study of newer antidepressant concentrations in cord and maternal serum and possible effects in the neonate. Int J Neuropsychopharmacol 2004 Sep;7:329–334.

47. Rampono J, Simmer K, Ilett KF, Hackett LP, Doherty DA, Elliot R, et al.: Placental transfer of SSRI and SNRI antidepressants and effects on the neonate. Pharmacopsychiatry 2009;42:95–100.

48. Altar CA, Hornberger J, Shewade A, Cruz V, Garrison J, Mrazek D: Clinical validity of cytochrome P450 metabolism and serotonin gene variants in psychiatric pharmacotherapy. Int Rev Psychiatry 2013 Oct;25:509–33.

49. Myllynen P, Pasanen M, Vähäkangas K: The fate and effects of xenobiotics in human placenta. Expert Opin Drug Metab Toxicol 2007;3:331–346.

50. Myllynen P, Immonen E, Kummu M, Vähäkangas K: Developmental expression of drug metabolizing enzymes and transporter proteins in human placenta and fetal tissues. Expert Opin Drug Metab Toxicol 2009 Dec;5:1483–1499.

51. Storvik M, Huuskonen P, Pehkonen P, Pasanen M: The unique characteristics of the placental transcriptome and the hormonal metabolism enzymes in placenta. Reprod Toxicol 2014;47:9–14.

52. Hemeryck A, Belpaire FM: Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update. Curr Drug Metab 2002;3:13–37.

53. Hiemke C, Härtter S: Pharmacokinetics of selective serotonin reuptake inhibitors. Pharmacol Ther 2000;85:11–28.

54. Sangkuhl K, Sting JC, Turpeinen M, Altman RB, Klein TE: PharmGKB summary: venlafaxine pathway. Pharmacogenet Genomics 2012;24:62–72.

55. Knadler MP, Lobo E, Chappell J, Bergstrom R: Duloxetine: clinical pharmacokinetics and drug interactions. Clin Pharmacokinet 2011;50:281–294.

56. Pastoor D, Gobburu J: Clinical pharmacology review of escitalopram for the treatment of depression. Expert Opin Drug Metab Toxicol 2014;10:121–128.

57. Swen JJ, Wilting I, de Goede AL, Grandia L, Mulder H, Touw DJ, et al.: Pharmacogenetics: from bench to byte. Clin Pharmacol Ther 2008;83:781–787.

58. Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, et al.: Pharmacogenetics: from bench to byte-an update of guidelines. Clin Pharmacol Ther 2011;89:662–673.

59. Hicks JK, Bishop JR, Sangkuhl K, Müller DJ, Ji Y, Leckband SG, et al.: Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and CYP2C19 Genotypes and Dosing of Selective Serotonin Reuptake Inhibitors. Clin Pharmacol Ther 2015;98:127–134.

60. Probst-Schendzielorz K, Viviani R, Stingl JC: Effect of Cytochrome P450 polymorphism

on the action and metabolism of selective serotonin reuptake inhibitors. Expert Opin Drug Metab Toxicol 2015;11:1219–1232.

61. Ververs FFT, Voorbij HAM, Zwarts P, Belitser S V., Egberts TCG, Visser GHA, et al.: Effect of cytochrome P450 2D6 genotype on maternal paroxetine plasma concentrations during pregnancy. Clin Pharmacokinet 2009;48:677–683.

62. LLerena A, Dorado P, Berecz R, González AP, Peñas-LLedó EM: Effect of CYP2D6 and CYP2C9 genotypes on fluoxetine and norfluoxetine plasma concentrations during steady-state conditions. Eur J Clin Pharmacol 2004;59:869–873.

63. Scordo MG, Spina E, Dahl M-L, Gatti G, Perucca E: Influence of CYP2C9, 2C19 and 2D6 genetic polymorphisms on the steady-state plasma concentrations of the enantiomers of fluoxetine and norfluoxetine. Basic Clin Pharmacol Toxicol 2005 Nov;97:296–301.

64. Grasmäder K, Verwohlt PL, Rietschel M, Dragicevic A, Müller M, Hiemke C, et al.: Impact of polymorphisms of cytochrome-P450 isoenzymes 2C9, 2C19 and 2D6 on plasma concentrations and clinical effects of antidepressants in a naturalistic clinical setting. Eur J Clin Pharmacol 2004;60:329–336.

65. Burk O, Tegude H, Koch I, Hustert E, Wolbold R, Glaeser H, et al.: Molecular mechanisms of polymorphic CYP3A7 expression in adult human liver and intestine. J Biol Chem 2002;277:24280–24288.

66. Werk AN, Cascorbi I: Functional gene variants of CYP3A4. Clin Pharmacol Ther 2014;96:340–348.

67. Fanni D, Fanos V, Ambu R, Lai F, Gerosa C, Pampaloni P, et al.: Overlapping between CYP3A4 and CYP3A7 expression in the fetal human liver during development. J Matern Neonatal Med 2015;28:1291–1295. DOI: 10.3109/14767058.2014.951625.

68. Haas DM, Lehmann AS, Skaar T, Philips S, McCormick CL, Beagle K, et al.: The impact of drug metabolizing enzyme polymorphisms on outcomes after antenatal corticosteroid use. Am J Obstet Gynecol 2012;206:e17-24.

69. Hakkola J, Pasanen M, Purkunen R, Saarikoski S, Pelkonen O, Mäenpää J, et al.: Expression of xenobiotic-metabolizing cytochrome P450 forms in human adult and fetal liver. Biochem Pharmacol 1994;48:59–64.

70. Koukouritaki SB, Manro JR, Marsh SA, Stevens JC, Rettie AE, McCarver DG, et al.: Developmental expression of human hepatic CYP2C9 and CYP2C19. J Pharmacol Exp Ther 2004;308:965–974.

71. Treluyer JM, Jacqz-Aigrain E, Alvarez F, Cresteil T: Expression of CYP2D6 in developing human liver. Eur J Biochem 1991;202:583–588.

72. Stevens J, Marsh S, Zaya M, Regina K, Divakaran K, Le M, et al.: Developmental changes in human liver CYP2D6 expression. Drug Metab Dispos 2008;36:1587–1593.

73. Nishimura M, Naito S: Tissue-specific mRNA expression profiles of human ATP-binding cassette and solute carrier transporter superfamilies. Drug Metab Pharmacokinet 2005 Dec;20:452–477.

74. Syme MR, Paxton JW, Keelan JA: Drug Transfer and Metabolism by the Human Placenta. Clin Pharmacokinet 2004;43:487–514.

75. Prouillac C, Lecoeur S: The Role of the Placenta in Fetal Exposure to Xenobiotics : Importance of Membrane Transporters and Human Models for Transfer Studies. Drug Metab Dispos 2010;38:1623–1635.

76. Ceckova-Novotna M, Pavek P, Staud F: P-glycoprotein in the placenta: expression, localization, regulation and function. Reprod Toxicol 2006;22:400–410.

77. Vähäkangas K, Myllynen P: Drug transporters in the human blood-placental barrier. Br J Pharmacol 2009 Oct;158:665–678.

78. Ganapathy V, Prasad PD: Role of transporters in placental transfer of drugs. Toxicol Appl Pharmacol 2005 Sep 1;207:381–7.

79. Zhou SF: Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. Xenobiotica 2008 Jul;38:802–832.

80. Iqbal M, Audette MC, Petropoulos S, Gibb W, Matthews SG: Placental drug transporters and their role in fetal protection. Placenta 2012;33:137–142.

81. Staud F, Cerveny L, Ceckova M: Pharmacotherapy in pregnancy; effect of ABC and SLC transporters on drug transport across the placenta and fetal drug exposure. J Drug Target 2012 Nov;20:736–763.

82. Gil S, Saura R, Forestier F, Farinotti R: P-glycoprotein expression of the human placenta during pregnancy. Placenta 2005;26:268–270.

83. Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, Gibb W: Expression of the Multidrug Resistance P-Glycoprotein, (ABCB1 glycoprotein) in the Human Placenta Decreases with Advancing Gestation. Placenta 2006;27:602–609.

84. Daud ANA, Bergman JEH, Bakker MK, Wang H, Kerstjens-Frederikse WS, de Walle HEK, et al.: P-Glycoprotein-Mediated Drug Interactions in Pregnancy and Changes in the Risk of Congenital Anomalies: A Case-Reference Study. Drug Saf 2015;38:651–659.

85. Kerb R: Implications of genetic polymorphisms in drug transporters for pharmacotherapy. Cancer Lett 2006 Mar 8;234:4–33.

86. Leschziner GD, Andrew T, Pirmohamed M, Johnson MR: ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. Pharmacogenomics J 2007 Jun;7:154–79.

87. Cascorbi I, Haenisch S: Pharmacogenetics of ATP-binding cassette transporters and clinical implications. Methods Mol Biol 2010 Jan;596:95–121.

88. Ieiri I: Functional significance of genetic polymorphisms in P-glycoprotein (MDR1, ABCB1) and breast cancer resistance protein (BCRP, ABCG2). Drug Metab Pharmacokinet 2012 Jan;27:85–105.

89. Noordam R, Aarts N, Hofman A, van Schaik RHN, Stricker BH, Visser LE: Association between genetic variation in the ABCB1 gene and switching, discontinuation, and dosage of antidepressant therapy: results from the Rotterdam Study. J Clin Psychopharmacol 2013;33:546–550.

90. Kato M, Fukuda T, Serretti A, Wakeno M, Okugawa G, Ikenaga Y, et al.: ABCB1 (MDR1) gene polymorphisms are associated with the clinical response to paroxetine in patients with major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2008 Feb;32:398–404.

91. Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmöller J, Johne A, et al.: Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci U S A 2000 Mar 28;97:3473–8.

92. Hitzl M, Schaeffeler E, Hocher B, Slowinski T, Halle H, Eichelbaum M, et al.: Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). Pharmacogenetics 2004 May;14:309–18.

93. Hemauer SJ, Nanovskaya TN, Abdel-Rahman SZ, Patrikeeva SL, Hankins GD, Ahmed MS: Modulation of human placental P-glycoprotein expression and activity by MDR1 gene polymorphisms. Biochem Pharmacol 2010;79:921–925.

94. Takane H, Kobayashi D, Hirota T, Kigawa J, Terakawa N, Otsubo K, et al.: Haplotype-Oriented Genetic Analysis and Functional Assessment of Promoter Variants in the MDR1 (ABCB1) Gene. J Pharmacol Exp Ther 2004;311:1179–1187.

95. Bliek BJB, van Schaik RHN, van der Heiden IP, Sayed-Tabatabaei FA, van Duijn CM, Steegers EAP, et al.: Maternal medication use, carriership of the ABCB1 3435C > T polymorphism and the risk of a child with cleft lip with or without cleft palate. Am J Med Genet A 2009 Oct;149A:2088–92.

96. Obermann-Borst SA, Isaacs A, Younes Z, van Schaik RHN, van der Heiden IP, van Duyn CM, et al.: General maternal medication use, folic acid, the MDR1 C3435T polymorphism, and the risk of a child with a congenital heart defect. Am J Obstet Gynecol 2011 Mar;204:236.e1-8.

97. Hutson JR, Garcia-Bournissen F, Davis A, Koren G: The human placental perfusion model: a systematic review and development of a model to predict in vivo transfer of therapeutic drugs. Clin Pharmacol Ther 2011;90:67–76.

98. Kuo H-W, Liu CL, Tsou H-H, Liu S, Lin K-M, Lu S, et al.: CYP1A2 genetic polymorphisms are associated with early antidepressant escitalopram metabolism and adverse reactions R esearch A rticle. Pharmacogenomics 2013;14:1191–1201.

99. Lin K-M, Tsou H-H, Tsai I-J, Hsiao M-C, Hsiao C-F, Liu C-Y, et al.: CYP1A2 genetic polymorphisms are associated with treatment response to the antidepressant paroxetine. Pharmacogenomics 2010;11:1535–1543.

100. Mrazek DA, Biernacka JM, O'Kane DJ, Black JL, Cunningham JM, Drews MS, et al.: CYP2C19 variation and citalopram response. Pharmacogenet Genomics 2011;21:1–9.

101. Jin Y, Pollock BG, Frank E, Cassano GB, Rucci P, Müller DJ, et al.: Effect of age, weight, and CYP2C19 genotype on escitalopram exposure. J Clin Pharmacol 2010;50:62–72.

102. Rudberg I, Mohebi B, Hermann M, Refsum H, Molden E: Impact of the ultrarapid CYP2C19*17 allele on serum concentration of escitalopram in psychiatric patients. Clin Pharmacol Ther 2008;83:322–327.

103. de Vos A, van der Weide J, Loovers HM: Association between CYP2C19*17 and metabolism of amitriptyline, citalopram and clomipramine in Dutch hospitalized patients. Pharmacogenomics J 2011;11:359–367.

104. Huezo-Diaz P, Perroud N, Spencer EP, Smith R, Sim S, Virding S, et al.: CYP2C19 genotype predicts steady state escitalopram concentration in GENDEP. J Psychopharmacol 2012;26:398–407.

105. Hermann M, Hendset M, Fosaas K, Hjerpset M, Refsum H: Serum concentrations of venlafaxine and its metabolites O-desmethylvenlafaxine and N-desmethylvenlafaxine in heterozygous carriers of the CYP2D6*3,*4 or*5 allele. Eur J Clin Pharmacol 2008;64:483–487.

106. Whyte EM, Romkes M, Mulsant BH, Kirshne MA, Begley AE, Reynolds CF, et al.: CYP2D6 genotype and venlafaxine-XR concentrations in depressed elderly. Int J Geriatr Psychiatry 2006;21:542–549.

107. Sawamura K, Suzuki Y, Someya T: Effects of dosage and CYP2D6-mutated allele on plasma concentration of paroxetine. Eur J Clin Pharmacol 2004;60:553–557.

108. Lin KM, Chiu YF, Tsai IJ, Chen CH, Shen WW, Liu SC, et al.: ABCB1 gene polymorphisms are associated with the

severity of major depressive disorder and its response to escitalopram treatment. Pharmacogenet Genomics 2011 Apr;21:163–170.

109. Singh AB, Bousman CA, Ng CH, Byron K, Berk M: ABCB1 polymorphism predicts escitalopram dose needed for remission in major depression. Transl Psychiatry 2012;2:e198.

110. Fukui N, Suzuki Y, Sawamura K, Sugai T, Watanabe J, Inoue Y, et al.: Dose-dependent effects of the 3435 C>T genotype of ABCB1 gene on the steady-state plasma concentration of fluvoxamine in psychiatric patients. Ther Drug Monit 2007 Apr;29:185–189.

111. Nikisch G, Eap CB, Baumann P: Citalopram enantiomers in plasma and cerebrospinal fluid of ABCB1 genotyped depressive patients and clinical response: a pilot study. Pharmacol Res 2008;58:344–347.

112. Sarginson JE, Lazzeroni LC, Ryan HS, Ershoff BD, Schatzberg AF, Murphy Jr GM: ABCB1 (MDR1) polymorphisms and antidepressant response in geriatric depression. Pharmacogenet Genomics 2010 Aug;20:467–475.

113. de Klerk OL, Nolte IM, Bet PM, Bosker FJ, Snieder H, den Boer JA, et al.: ABCB1 gene variants influence tolerance to selective serotonin reuptake inhibitors in a large sample of Dutch cases with major depressive disorder. Pharmacogenomics J 2013 Aug;13:349–353.

114. Huang X, Yu T, Li X, Cao Y, Li X, Liu B, et al.: ABCB6, ABCB1 and ABCG1 genetic polymorphisms and antidepressant response of SSRIs in Chinese depressive patients. Pharmacogenomics 2013;14:1723–1730.

115. Gex-Fabry M, Eap CB, Oneda B, Gervasoni N, Aubry J-M, Bondolfi G, et al.: CYP2D6 and ABCB1 genetic variability: influence on paroxetine plasma level and therapeutic response. Ther Drug Monit 2008;30:474–482.

116. Velasquez JC, Goeden N, Bonnin A: Placental serotonin: implications for the developmental effects of SSRIs and maternal depression. Front Cell Neurosci 2013;7:1–7.

117. Lamers WH, Virágh S, Wessels A, Moorman AF, Anderson RH: Formation of the tricuspid valve in the human heart. Circulation 1995;91:111–21.

118. Nebigil CG, Choi D, Dierich A, Hickel P, Le Meur M, Messaddeq N, et al.: Serotonin 2B receptor is required for heart development. Proc Natl Acad Sci 2000;97:9508–9513.

119. Nebigil CG, Hickel P, Messaddeq N, Vonesch J-L, Douchet MP, Monassier L, et al.: Ablation of Serotonin 5-HT2B Receptors in Mice Leads to Abnormal Cardiac Structure and Function. Circulation 2001 Jun 19;103:2973–2979.

120. Sadler TW: Selective serotonin reuptake inhibitors (SSRIs) and heart defects: potential mechanisms for the observed associations. Reprod Toxicol 2011 Dec;32:484–489.

121. Bajolle F, Zaffran S, Bonnet D: Genetics and embryological mechanisms of congenital heart diseases. Arch Cardiovasc Dis 2009;102:59–63.

122. Kekuda R, Leibach FH, Furesz TC, Smith CH, Ganapathy V: Polarized distribution of interleukin-1 receptors and their role in regulation of serotonin transporter in placenta. J Pharmacol Exp Ther 2000 Mar;292:1032–41.

123. Oberlander TF: Fetal serotonin signaling: Setting pathways for early childhood development and behavior. J Adolesc Heal 2012;51:S9–S16.

124. Olivier JDA, Akerud H, Kaihola H, Pawluski JL, Skalkidou A, Högberg U, et al.: The effects of maternal depression and maternal

selective serotonin reuptake inhibitor exposure on offspring. Front Cell Neurosci 2013;7:1–15.

125. Olivier JDA, ÃKerud H, Skalkidou A, Kaihola H, Sundström-Poromaa I: The effects of antenatal depression and antidepressant treatment on placental gene expression. Front Cell Neurosci 2015;8:1–17.

126. Noorlander CW, Ververs FFT, Nikkels PGJ, van Echteld CJA, Visser GHA, Smidt MP: Modulation of serotonin transporter function during fetal development causes dilated heart cardiomyopathy and lifelong behavioral abnormalities. PLoS One 2008 Jan;3:e2782.

127. Yavarone MS, Shuey DL, Tamir H, Sadler TW, Lauder JM: Serotonin and cardiac morphogenesis in the mouse embryo. Teratology 1993;47:573–584.

128. Oberlander TF, Bonaguro RJ, Misri S, Papsdorf M, Ross CJ, Simpson EM: Infant serotonin transporter (SLC6A4) promoter genotype is associated with adverse neonatal outcomes after prenatal exposure to serotonin reuptake inhibitor medications. Mol Psychiatry 2008 Jan;13:65–73.

129. Zhang H, Smith GN, Liu X, Holden JJA: Association of MAOA, 5-HTT, and NET promoter polymorphisms with gene expression and protein activity in human placentas. Physiol Genomics 2010 Jun;42:85–92.

130. Kraft JB, Slager SL, McGrath PJ, Hamilton SP: Sequence analysis of the serotonin transporter and associations with antidepressant response. Biol Psychiatry 2005 Sep;58:374–381.

131. Kroeze Y, Zhou H, Homberg JR: The genetics of selective serotonin reuptake inhibitors. Pharmacol Ther 2012 Dec;136:375–400.

132. Wilkie MJ, Smith G, Day RK, Matthews K, Smith D, Blackwood D, et al.: Polymorphisms in the SLC6A4 and HTR2A genes influence treatment outcome following antidepressant therapy. Pharmacogenomics J 2009 Feb;9:61–70.

133. Kishi T, Yoshimura R, Kitajima T, Okochi T, Okumura T, Tsunoka T, et al.: HTR2A is associated with SSRI response in major depressive disorder in a Japanese cohort. Neuromolecular Med 2010 Sep;12:237–42.

134. McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, et al.: Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. Am J Hum Genet 2006 May;78:804–14.

135. Peters EJ, Slager SL, Jenkins GD, Reinalda MS, Garriock HA, Shyn SI, et al.: Resequencing of serotonin-related genes and association of tagging SNPs to citalopram response. Pharmacogenet Genomics 2009;19:1–10.

136. Choi M-J, Kang R-H, Ham B-J, Jeong H-Y, Lee M-S: Serotonin receptor 2A gene polymorphism (-1438A/G) and short-term treatment response to citalopram. Neuropsychobiology 2005 Jan;52:155–162.

137. Kato M, Fukuda T, Wakeno M, Fukuda K, Okugawa G, Ikenaga Y, et al.: Effects of the serotonin type 2A, 3A and 3B receptor and the serotonin transporter genes on paroxetine and fluvoxamine efficacy and adverse drug reactions in depressed Japanese patients. Neuropsychobiology 2006;53:186–195.

138. Villafuerte SM, Vallabhaneni K, Sliwerska E, McMahon FJ, Young EA, Burmeister M: SSRI response in depression may be influenced by SNPs in HTR1B and HTR1A. Psychiatr Genet 2009 Dec;19:281–91.

139. Hong CJ, Chen TJ, Yu YW, Tsai SJ: Response to fluoxetine and serotonin 1A receptor (C-1019G) polymorphism in Taiwan Chinese major depressive disorder. Pharmacogenomics J 2006;6:27–33.

140. Yu YW-Y, Tsai S-J, Liou Y-J, Hong C-J, Chen T-J: Association study of two serotonin 1A receptor gene polymorphisms and fluoxetine treatment response in Chinese major depressive disorders. Eur Neuropsychopharmacol 2006 Oct;16:498–503.

141. Serretti A, Artioli P, Lorenzi C, Pirovano A, Tubazio V, Zanardi R: The C(-1019) G polymorphism of the 5-HT1A gene promoter and antidepressant response in mood disorders: preliminary findings. Int J Neuropsychopharmacol 2004;7:453–460.

142. Sugai T, Suzuki Y, Sawamura K, Fukui N, Inoue Y, Someya T: The effect of 5-hydroxytryptamine 3A and 3B receptor genes on nausea induced by paroxetine. Pharmacogenomics J 2006;6:351–356.

143. Tanaka M, Kobayashi D, Murakami Y, Ozaki N, Suzuki T, Iwata N, et al.: Genetic polymorphisms in the 5-hydroxytryptamine type 3B receptor gene and paroxetine-induced nausea. Int J Neuropsychopharmacol 2008;11:261–267.

144. Ori M, De Lucchini S, Marras G, Nardi I: Unraveling new roles for serotonin receptor 2B in development: Key findings from Xenopus. Int J Dev Biol 2013;57:707–714.

145. Gelb BD, Chung WK: Complex Genetics and the Etiology of Human Congenital Heart Disease. Cold Spring Harb Perspect Med 2014;4:1–12.

146. Kaihola H, Olivier J, Poromaa IS, Åkerud H: The effect of antenatal epression and selective serotonin reuptake inhibitor treatment on nerve growth factor signaling in human placenta. PLoS One 2015;10:e0116459.doi:10.1371/journal.pone.0116459.

147. Lee SH, Choi TK, Lee E, Seok JH, Lee HS, Kim SJ: Serotonin transporter gene polymorphism associated with short-term treatment response to venlafaxine. Neuropsychobiology 2010 Aug;62:198–206.

148. Perlis RH, Mischoulon D, Smoller JW, Wan YJ, Lamon-Fava S, Lin KM, et al.: Serotonin transporter polymorphisms and adverse effects with fluoxetine treatment. Biol Psychiatry 2003 Nov;54:879–883.

149. Hu X-Z, Rush AJ, Charney D, Wilson AF, Sorant AJM, Papanicolaou GJ, et al.: Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. Arch Gen Psychiatry 2007 Jul;64:783–792.

150. Maron E, Tammiste A, Kallassalu K, Eller T, Vasar V, Nutt DJ, et al.: Serotonin transporter promoter region polymorphisms do not influence treatment response to escitalopram in patients with major depression. Eur Neuropsychopharmacol 2009 Jun;19:451–456.

151. Murphy GM, Hollander SB, Rodrigues HE, Kremer C, Schatzberg AF: Effects of the serotonin transporter gene promoter polymorphism on mirtazapine and paroxetine efficacy and adverse events in geriatric major depression. Arch Gen Psychiatry 2004;61:1163–1169.

152. Smits K, Smits L, Peeters F, Schouten J, Janssen R, Smeets H, et al.: Serotonin transporter polymorphisms and the occurrence of adverse events during treatment with selective serotonin reuptake inhibitors. Int Clin Psychopharmacol 2007;22:137–143.

153. Staeker J, Leucht S, Laika B, Steimer W: Polymorphisms in serotonergic pathways influence the outcome of antidepressant therapy in psychiatric inpatients. Genet Test Mol Biomarkers 2014 Jan;18:20–31.

chapter 7

Prenatal exposure to serotonin reuptake inhibitors and congenital heart anomalies:

An exploratory gene-environment interaction study

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Submitted

ABSTRACT

Background: Prenatal use of serotonin reuptake inhibitors (SRIs) was previously associated with congenital heart anomalies (CHA). We aimed to explore whether pharmacogenetics has a role in this fetal outcome.

Methods: A total of 33 case-mother dyads and 2 mother-only (children deceased) cases registered in EUROCAT Northern Netherlands were included in a case-only study. Of these, five case-mother dyads and two mother-only (children deceased) were exposed to SRIs in the first trimester of pregnancy. Ten genes encoding enzymes or proteins important in determining fetal exposure to SRIs or its mechanism were selected: genes coding for CYP450 enzymes (*CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6)*, a P-glycoprotein coding gene (*ABCB1*), a serotonin transporter gene (*SLC6A4*) and serotonin receptor genes (*HTR1A*, *HTR1B*, *HTR2A*, and *HTR3B*). All subjects were genotyped for 58 genetic variations in these genes.

Results: Among exposed cases, several polymorphisms tended to be associated with an increased risk of CHA: *ABCB1* rs1128503, *SLC6A4* 5-HTTLPR and 5-HTTVNTR, *HTR1A* rs1364043, *HTR1B* rs6296 & rs6298 and *HTR3B* rs1176744. However, the sample sizes of this exploratory study were limited and our results did not reach statistical significance.

Conclusion: This is the first study to explore the CHA risk associated with potential gene-environment interaction between pharmacogenetic determinants and SRI use. We did find indications of a role for serotonin receptor polymorphisms in fetuses exposed to SRIs that warrants further investigation.

INTRODUCTION

One of the most prescribed antidepressant groups is the selective serotonin reuptake inhibitors (SSRIs), with up to four in 100 pregnant women being prescribed with this group of antidepressants (1-3). SSRIs are generally well tolerated with the exception of concerns about the increased risk of fetal congenital anomalies following prenatal exposure to these drugs. Following the US Food and Drug Administration (FDA) warning about this risk in 2005, many studies have been performed to elucidate the magnitude and effect of this association. However, the results of these studies have been inconsistent. Meta-analyses by two groups of researchers reported around a 40% increase in the risk of fetal congenital heart anomalies (CHA) following prenatal exposure to paroxetine (4-6), but a similar risk increment was not found for all SSRIs combined (7). Because clinical trials are not an option to measure the risk of an exposure during pregnancy, most studies were done retrospectively using data from pregnancy and/or prescription registries. The conflicting study results impede decision-making among clinicians on a safe and effective therapy for their patients, and best practice at present is to assess individual risk factors before any treatment recommendation.

We previously identified several genes that might be important in the metabolism and mechanism of action of SRIs that may also potentially play a role in the development of SRI-related CHA (8). Several polymorphisms of metabolic enzymes (CYP1A2, CYP2C9, CYPC19 and CYP2D6) were reported to affect the pharmacokinetics and the risk of side effects of SRIs (9,10). P-glycoprotein (P-gp) expressed in the placenta plays a role in limiting fetal exposure to SRIs, and several single nucleotide polymorphisms (SNPs) were found to reduce P-gp function (11). In addition, a number of polymorphisms of the serotonin transporter (SERT) and the serotonin receptors these genes were associated with variation in the clinical response to SRIs and the severity of side effects (12-14).

We therefore aimed to explore the genetic variations that may be involved in fetal exposure to SRIs, and their mechanism of action, to further understand why some children exposed to SRIs in the first trimester of pregnancy develop CHA while others do not. Our objective was to determine the effect of the gene x environment (G x E) interaction between pharmacogenetic predictors of the SRIs and prenatal exposure to these drugs on the risk of CHA.

METHODS

Study design and patient sampling

We performed an exploratory G x E interaction study using case-only design. This design can detect the effect of genotype and exposure in a group of cases when the disease is rare. One of the assumptions made is that the genotype and environment are independent of each other (15-17). The study population includes children with CHA registered in the EUROCAT Northern Netherlands (NNL) database, a population-based birth defect registry covering the three northern provinces of the Netherlands. EUROCAT NNL registers fetuses or children diagnosed with major congenital anomalies before or after birth, and up to 10 years old, upon consent of their parents. For cases registered up to 2001, the types of CHA were classified according to the EUROCAT Subgroup of Congenital Anomalies version 2012 (18) and the International Classification of Diseases (ICD) coding system 9th revision. For cases registered from 2002 onwards, the ICD coding system 10th revision was used for classification. We included only major CHA cases, either as single heart anomalies, as part of complex heart anomalies (including cardiovascular anomalies), or as part of complex anomalies involving other organ systems. Diagnosis codes included were ICD9 745-746, 7470-7474 (excluding 74550) and ICD10 Q20-Q26 (excluding Q2111).

Cases born between January 1, 1997 and December 31, 2013 were eligible for this study. Exclusion criteria were: 1) cases with genetic disorders, including chromosomal anomalies, microdeletions, monogenic disorders and with known teratogenic causes; 2) case mothers with a previous history of a malformed child or history unknown; and 3) cases in which the mother never used any medication during pregnancy in order to reduce the selection bias of including mothers among the unexposed group who were generally 'healthy'. Cases were invited to participate in this study via the Pediatric Cardiology Clinic, University Medical Center Groningen (UMCG), and were asked to provide DNA samples. This study received a waiver from ethical clearance consideration by the Medical Ethical Committee of the UMCG.

Drug exposure

Exposed cases were defined as CHA cases whose mothers had used at least one of the following SRIs (ATC codes) at some point between 30 days before conception and 90 days of gestation: fluoxetine (N06AB03), citalopram (N06AB04), paroxetine (N06AB05), sertraline (N06AB06), fluvoxamine (N06AB08), escitalopram (N06AB10), venlafaxine (N06AX16) and duloxetine (N06AX21). The information on drug use in EUROCAT NNL was obtained primarily via pharmacy records, upon consent of the mother, and later verified by telephone interviews to ensure the validity

of the information obtained. The unexposed cases were CHA cases whose mothers had used any drugs other than SRIs during pregnancy. Variables like smoking during the pregnancy, alcohol intake during the pregnancy, maternal medical history and folic acid supplementation were obtained from a questionnaire given upon registration with EUROCAT NNL.

Selection of candidate genes and SNPs

We selected 10 genes that encode enzymes or proteins important in determining fetal exposure to SRIs: the CYP450 enzymes (*CYP1A2*, *CYP2C9, CYP2C19* and *CYP2D6*), P-gp (*ABCB1*), SERT (*SLC6A4*), and serotonin receptors (*HTR1A, HTR1B, HTR2A, HTR3B*). The CYP450 metabolic enzymes are involved in the pharmacokinetics of SRIs and influence the drug concentration in the maternal circulation. Since all the SRIs examined in this study are substrates of P-gp, changes in P-gp expression or activity may alter the fetal exposure to SRIs (19,20). SRIs inhibit the uptake of serotonin (5-HT) through the SERT, and 5-HT signals through serotonin receptors. A normal 5-HT signaling is important for normal development of fetal heart cells (21).

For the *CYP1A2*, *CYP2C9*, *CYP2C19* and *CYP2D6* genes, we selected 37 SNPs with known phenotypes of either "ultrarapid metabolizer", "rapid metabolizer", "extensive metabolizer", "intermediate metabolizer" or "poor metabolizer" " (http://www.cypalleles.ki.se/). The selection of polymorphisms in *ABCB1*, *SLC6A4* and serotonin receptor genes was based on their clinical effects on SRIs treatment: 8 SNPs in *ABCB1*, 2 repeat markers in *SLC6A4*, 2 SNPs in *HTR1A,* 2 SNPs in *HTR1B,* 5 SNPs in *HTR2A,* and 2 SNPs in *HTR3B* (**Appendix 6.1**) (8,14,22,23). SNPs with call rates of <90% were excluded from the analysis.

DNA collection

An invitation letter and package was sent to the mother of each exposed case, followed by a reminder letter after four weeks, if necessary. Once written informed consent was received from the mothers (and children), we sent them the sample collection kit including cytobrushes to collect buccal cell samples (Isohelix SK-1 swab kits with Isohelix Dri-capsules, Cell Projects Ltd, UK). A clear instruction on how to use the sample collection kit was provided, together with the link to an instruction video (in Dutch). Mothers (and children) were asked to return the cytobrushes, with a silica gel enclosed, to the researchers in prepaid mail envelopes. A reminder letter was sent if we did not receive the samples after four weeks. Each collection tube containing the samples was labelled with the identifier code and with 'Mother' or 'Child'. DNA samples received from the exposed cases were labelled and stored until

they were genotyped. The DNA from exposed cases was extracted from the buccal cells using Isohelix DNA isolation kit (DDK-50/DDK-3, Cell Projects Ltd, UK). For the unexposed cases, DNA samples were retrieved from CHA patients from the Department of Genetics, UMCG who consented with the use of residual materials. The DNA was obtained from blood and the isolation process was performed in the same facility as the samples from exposed cases.

Genotyping

SNP genotyping for *CYPs*, *ABCB1* and *HTR* genes was performed using 10 ng of DNA samples using the iPLEX® Gold platform (Agena Bioscience GmbH, Hamburg, Germany) according to the standard protocol. The region of interest was amplified by polymerase chain reaction (PCR) using gene-specific primers, followed by single base extension using the iPLEX Gold cocktail of primer, enzyme, buffer and terminator nucleotides, resulting in extended fragments with a specific mass for each allele. The mass was detected by the MassARRAY® System and genotype calling was performed using the MassARRAY® Typer Analyzer 4.0 software tools (Agena Bioscience GmbH or Sequenom, Hamburg, Germany). Manual inspection and adjustment of the genotype classifications was also performed by authors on all SNPs with call rates of less than 90%. For the *SLC6A4* repeat markers, the regions of 5-HTTLPR and 5-HTTVNTR were amplified by PCR using specific primers. Amplified DNA fragments were separated by electrophoresis: 5-HTTLPR long and short alleles (530 bp and 486 bp, respectively) and 5-HTTVNTR STin2.9, STin2.10 and STin2.12 (250 bp, 271 bp, and 302 bp, respectively).

Phenotype and genotype scoring

The genotypes of CYP enzyme polymorphisms were grouped into phenotypes that depict the functionality of the enzymes (i.e. normal metabolizer, poor metabolizer or rapid metabolizer etc.), and were reported according to the standardized terms from the Clinical Pharmacogenomics Implementation Consortium (24,25). Since the CYP enzymes in the fetus are not fully developed during the first trimester, only the genotype from the mothers was analyzed.

The risk of CHA was determined for each genetic variation of the *ABCB1*, *HTR1A*, *HTR1B*, *HTR2A* and *HTR2B* genes using a recessive model and for the *SLC6A4* gene using a dominant model, based on the number of exposed cases to perform the analysis. To further explore the cumulative effect of *ABCB1* SNPs, we calculated a genetic score per individual based on the number of risk alleles present as done previously (26-28). The score is associated with the transport of SRIs through P-gp. In the mother, P-gp is expressed in the intestine, liver and kidney where it helps to

eliminate substrate drugs, while P-gp in the placenta limits drug transport into the fetal circulation. A maternal *ABCB1* genotype encoding for reduced P-gp function increases the plasma drug concentration available for transfer through the placenta, while the same genotype in the fetus increases the transfer of the drug into the fetus. Seven SNPs in the *ABCB1* gene previously associated with reduced expression or function were included in the scoring: rs1045642, rs1128503, rs1882478, rs2032582, rs2235040, rs4148739 and rs9282564. The risk alleles can occur in a homozygous or heterozygous form; therefore each individual could have 0, 1, or 2 alleles for each SNP, resulting in a cumulative risk score up to 14. For the *SLC6A4* 5-HTTLPR and 5-HTTVNTR polymorphisms, the cumulative score was up to 4.

Statistical analysis

Deviations from Hardy-Weinberg equilibrium were tested using Pearson's chi-square test. To test for the effect of pharmacogenetic predictors (genotype) and prenatal exposure to SRIs (environment) on the risk of fetal CHA, we determined the departure from multiplicative interaction between gene and environment using multivariable logistic regression and expressed with interaction odds ratio (OR) and 95% confidence interval (CI). An OR of more than 1 indicates that the presence of both pharmacogenetic predictors and SRI-use increases the risk of CHA.

RESULTS

Case sampling

From 2,172 CHA cases born between 1997 and 2013 and registered in EUROCAT NNL, we selected 1,383 cases that matched the inclusion criteria (**Figure 1**). For the exposed cases, twenty-four case mothers were invited to participate in the study, and eight case-mother dyads gave their consent. For cases under the age of 12, written informed consent was obtained from their mothers. The DNA samples were available for five exposed dyads and two mothers-only (with deceased child) cases; four exposed case-mother dyads and two mothers-only cases provided their DNA samples, and the samples of one case-mother dyad were retrieved from the clinical diagnostic laboratory. The number of unexposed cases was decided to be four times the number of exposed cases, therefore 28 unexposed case-mother dyads were randomly selected from the available DNA samples.

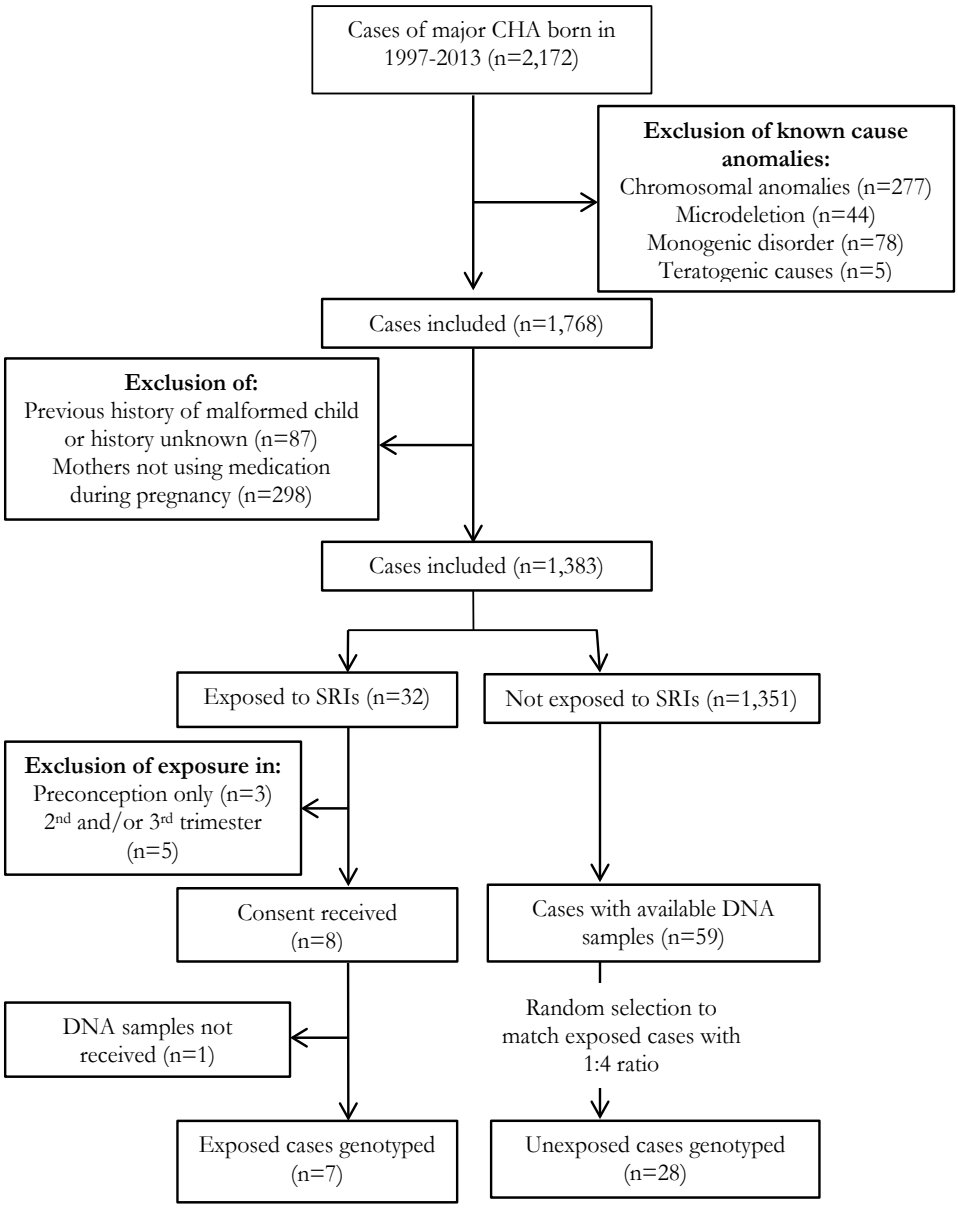

Figure 1: Case sampling

The characteristics of all cases and mothers can be found in **Table 1**. The majority of our cases and case mothers are Caucasians (91.4%). The SRIs used by the case-mothers were paroxetine (3), fluoxetine (2), venlafaxine (1), and paroxetine and venlafaxine (1). Among all cases, the types of medications used in the first trimester are: antiemetics (6), analgesics (6), hormone preparations (5), antacids (4), laxatives (4), antibiotics (3), antihistamines (2), thyroid preparation (1), cholesterol lowering agent (1) and cough preparation (1).

Genotyping

A total of 65 DNA samples were obtained and genotyped: 12 samples from the exposed cases (5 children and 7 mothers) and 53 samples from the unexposed cases (28 children and 25 mothers). Genotype and allele frequencies for all case-mother dyads are listed in **Table 2**. Out of 58 polymorphisms analyzed, 5 SNPs (*CYP2D6 *4, *6, *8,*17, *41*) failed to be genotyped. Out of 53 SNPs, 4 SNPs (*CYP2D6*2, *9, *11* and **12*) had allele calling rates of less than 90% and the genotype frequency for *HTR2A* rs7997012 was not in Hardy Weinberg equilibrium (p=0.03). Due to the different call rates among SNPs, the number of case-mother dyads differed for each G x E interaction analysis.

CYP enzyme and P-glycoprotein phenotypes, SRI exposure and CHA

The interaction between maternal CYP enzyme phenotypes and SRI exposure does not indicate an effect on the risk of CHA. Among cases exposed to paroxetine (n=4), all of the case-mothers were normal CYP2D6 metabolizers (**Appendix 6.2**). Two cases were exposed to venlafaxine, and one of them is an intermediate CYP2C19 metabolizer. Fluoxetine was used by two case-mothers who are both normal CYP2C9 and CYP2D6 metabolizers. Therefore, we cannot determine the effect of metabolic enzyme phenotypes on the risk of CHA associated with the use of SRIs among our case-mothers.

Table 1: Characteristics of case-mother dyads included in the study (N=35)

Characteristics	n	%
Child sex		
Boy	27	77.1
Girl	8	22.9
Year of birth		
2003-2007	24	68.6
2008-2013	11	31.4
First pregnancy	8	22.9
Types of birth		
Live birth	33	94.3
Termination of pregnancy	2	5.7
Types of CHA		
Single	22	62.9
Complex	13	37.1
Subtypes of CHA**		
Cardiac cambers and connections, ICD10 Q20	5	14.3
Cardiac septa, ICD10 Q21	11	31.4
Pulmonary and tricuspid valves, ICD10 Q22	2	5.7
Aortic and mitral valves, ICD10 Q23	22	62.9
Great arteries, ICD10 Q25	12	34.3
Maternal age at delivery, mean years (range)	31	24-39
Maternal education level		
Low	2	5.7
Middle	18	51.4
High	15	42.9
Folic acid use during pregnancy	33	94.3
Smoking during first trimester	6	17.1
Alcohol intake in the first trimester	14	40.0
Medication use in the first trimester		
SRIs	7	20
Other medication***	23	65.7
Maternal medical history		
Gestational diabetes	3	8.6
Congenital anomalies	4	11.4
Chronic disease	6	17.1

CHA: congenital heart anomalies; SRIs: serotonin reuptake inhibitors; **more than one subtype is counted for cases of complex CHA; ***other than SRIs (for exposed group) and folic acid/supplements (for unexposed group); within 30 days before conception and 90 days in the first trimester

For *ABCB1*, there is no indication of changes in the risk of CHA with any of the *ABCB1* SNPs in the mothers and the children, except possibly for maternal rs1128503 (**Table 3**). However, the sample size was too small to reach statistical significance. For the maternal genotype, the mean score among the exposed case mothers was 3.9 ± 0.7, while the mean scores of the unexposed case mothers was 4.3 ± 1.9 (p=0.41). The distribution of the genetic scores of the exposed and unexposed cases is shown in Figure 2. The mean genetic score of the exposed cases (children) was 5.0 ± 1.9 and 4.4 ± 1.8 among the unexposed cases (p=0.47).

Serotonin transporter and receptor polymorphisms, SRI exposure and CHA

The LL genotype of the *SLC6A4* 5-HTTLPR and 12/12 genotype of the 5-HTTVNTR indicated an increase in the risk of CHA among cases exposed to SRIs, although not significant (**Table 3**). The mean genetic scores of the exposed mothers tended to be higher than the unexposed mothers (2.5 ± 0.8 versus 1.88 ± 0.7, respectively; p=0.061) (**Figure 3**). Meanwhile, the mean genetic scores of the exposed and unexposed cases (children) were comparable (2.4 ± 0.5 and 2.18 ± 0.8, respectively; p=0.57).

For fetal 5-HT receptors, the SNPs in *HTR1A*, *HTR1B* and *HTR3B* showed increases in the interaction OR, although none achieved statistical significance (**Table 3**). We then calculated the genetic scores for these SNPs, which include *HTR1A* rs1364043, *HTR1B* rs6296, rs6298 and *HTR3B* rs1176744 (maximum score of 8).The mean genetic score for exposed cases tended to be higher as compared to unexposed cases (3.4 ± 2.2 versus 1.9 ± 1.6, respectively; p=0.065), and the distribution was skewed towards higher genetic scores (**Figure 4**).

Table 2: Genotype frequency of study SNPs in case-mother dyad samples (N=65)

Gene/ SNPs	rs number	wt/vt allele	wt/wt	wt/vt	vt/vt	NA	Allele calling rate	Variant allele fq	Variant allele fq (European)*	HWE p value
CYP1A2										
	rs2069521	G/A	62	1	0	2	96.9	0.01	0.02	0.95
	rs2069526	T/G	62	1	0	2	96.9	0.01	0.02	0.95
	rs4646425	C/T	62	1	0	2	96.9	0.01	0.02	0.95
	rs4646427	T/C	63	1	0	1	98.5	0.01	0.02	0.95
	rs2472304	G/A	4	27	32	2	96.9	0.72	0.6	0.59
	rs2470890	C/T	5	26	33	1	98.5	0.72	0.6	0.97
CYP2C9										
***2**	rs1799853	C/T	65	0	0	0	100	0	0.12	NA
***3**	rs1057910	A/C	57	7	0	1	98.5	0.05	0.07	0.64
***4**	rs56165452	T/A	62	1	0	2	96.9	0.01	0†	0.95
***6**	rs9332131	A/DEL	62	0	0	3	95.4	0	0	NA
***5**	rs28371686	C/G	65	0	0	0	100	0	0	NA
***8**	rs7900194	G/A	64	0	0	1	98.5	0	0	NA
***11**	rs28371685	C/T	65	0	0	0	100	0	0	NA
***13**	rs72558187	T/C	65	0	0	0	100	0	0	NA
***15**	rs72558190	C/A	65	0	0	0	100	0	0†	NA
CYP2C19										
***2**	rs4244285	G/A	51	12	2	0	100	0.12	0.15	0.24
***3**	rs4986893	G/A	65	0	0	0	100	0	0	NA
***4**	rs28399504	A/G	59	1	0	5	92.3	0.01	0	0.95
***5**	rs56337013	C/T	65	0	0	0	100	0	0†	NA

***6**	rs72552267	G/A	65	0	0	0	100	0	0	NA
***7**	rs72558186	T/A	64	0	0	1	98.5	0	0†	NA
***8**	rs41291556	T/C	64	0	0	1	98.5	0	0	NA
***9**	rs17884712	G/C	65	0	0	0	100	0	0	NA
***10**	rs6413438	C/T	65	0	0	0	100	0	0	NA
***17**	rs12248560	C/T	35	23	3	4	93.8	0.24	0.22	0.75
CYP2D6										
***2**	rs16947	G/A	27	18	9	11	83.1	0.33	0.34	0.06
***3A**	rs35742686	A/DEL	61	0	0	4	93.8	0	0.02	NA
***7**	rs5030867	A/C	64	0	0	1	98.5	0	0	NA
***9**	rs5030656	AAG/ DEL	13	0	0	52	20	0	0.03	NA
***10**	rs1065852	C/T	39	22	4	0	100	0.23	0.2	0.71
***11**	rs5030863	G/C	44	0	0	21	67.7	0	NA	NA
***12**	rs5030862	G/A	56	0	0	9	86.2	0	0	NA

Fq, frequency; wt, wild type; vt, variant; HWE, Hardy Weinberg equilibrium; NA, not available; * allele fq of the European population (http://www.ncbi.nlm.nih.gov/SNP/ , http://www.ensembl.org/index.html); † allele frequency of population worldwide.

Table 2 (cont.): Genotype frequency of study SNPs in case-mother dyad samples (N=65)

Gene/ SNPs	rs number	wt/vt allele	wt/ wt	wt/vt	vt/vt	NA	Allele calling rate	Variant allele fq	Variant allele fq (European)*	HWE p value
ABCB1	rs1128503	C/T	19	29	14	3	95.4	0.46	0.42	0.65
	rs2032582	G/T/A	19	29	14 (TT), 1 (TA)	2	96.9	0.46(T),0.01(A)	0.41 (T), 0.02(A)	0.71
	rs1045642	C/T	13	27	23	2	96.9	0.58	0.52	0.34
	rs2235040	G/A	43	19	1	2	96.9	0.17	0.13	0.5
	rs4148739	A/G	43	18	1	3	95.4	0.16	0.13	0.57
	rs1882478	G/A	40	20	2	3	95.4	0.19	0.26	0.79
	rs9282564	A/G	46	14	2	3	95.4	0.15	0.08	0.48
	rs10256836	G/C	3	26	34	2	96.9	0.75	0.3	0.48
SLC6A4										
5HTTLPR	rs4795541	S/L	18	32	13	2	96.9	0.46 (L)	0.40	0.86
5HTTVNT	rs57098334	STin2.9, 10,12	-	4 (9/10), 5 (9/12), 22 (10/12)	8 (10/10), 25 (12/12)	1	98.5	0.07 (9) 0.33 (10) 0.6 (12)	0.47 (10)	0.69
HTR1A	rs1364043	A/C	38	21	4	2	96.9	0.23	0.21	0.64
	rs6295	G/C	16	34	14	1	98.5	0.48	0.54	0.61
HTR1B	rs6296	G/C	30	24	9	2	96.9	0.33	0.74	0.26
	rs6298	C/T	30	24	9	2	96.9	0.33	0.26	0.26
HTR2A	rs7997012	C/T	25	23	16	1	98.5	0.43	0.43	0.03
	rs6313	C/T	26	29	8	2	96.9	0.36	0.44	0.98
	rs6314	C/T	51	12	0	2	96.9	0.09	0.08	0.4
	rs1928040	C/T	16	30	17	2	96.9	0.38	0.49	0.71
	rs6311	G/A	26	28	10	1	98.5	0.37	0.44	0.59
HTR3B	rs1176744	A/C	37	23	3	2	96.9	0.23	0.31	0.81
	rs3831455	TCC/DEL	63	0	0	2	96.9	0	NA	NA

Fq, frequency; wt, wild type; vt, variant; HWE, Hardy Weinberg equilibrium; NA, not available; S, short allele; L, long allele; * allele fq of the European population (http://www.ncbi.nlm.nih.gov/SNP/ , http://www.ensembl.org/indexhtml)

Table 3: The G x E interaction effect of several SRIs pharmacogenetic predictors on the risk of CHA

SNPs/Genetic variations	Case mothers with variant alleles, n (%)			Cases with variant alleles, n (%)		
	Exposed n (%) N=7	Unexposed n (%) N=25	OR (95% CI)	Exposed n (%) N=5	Unexposed n (%) N=28	OR (95% CI)
ABCB1						
rs1045642	7 (100.0)	19 (76.0)	0.94 (0.48-1.82)	3 (60.0)	21 (75.0)	0.43 (0.058-3.14)
rs1128503	6 (85.7)	14 (56.0)	3.86 (0.4-37.58)	3 (60.0)	20 (71.4)	0.53 (0.072-3.82)
rs1882478	2 (28.6)	9 (36.0)	0.64 (0.13-3.06)	2 (40.0)	9 (32.1)	0.80 (0.32-1.99)
rs2032582	6 (58.7)	15 (60.0)	0.95 (0.52-1.76)	3 (60.0)	20 (71.4)	0.52 (0.086-3.59)
rs2235040	2 (28.6)	8 (32.0)	0.71 (0.19-2.67)	2 (40.0)	8 (28.6)	0.89 (0.38-2.10)
rs4148739	2 (28.6)	7 (28.0)	0.71 (0.24-2.09)	2 (40.0)	8 (28.6)	0.89 (0.38-2.10)
rs9282564	0	7 (28.0)	-	1 (20.0)	8 (28.6)	0.58 (0.07-5.08)
rs10256836	7 (100)	22 (88.0)	0.84 (0.32-2.18)	5 (100)	26 (92.9)	-
SLC6A4	*[N=6]*	*[N=24]*		*[N=5]*	*[N=28]*	
5-HTTLPR (*LL*)	2 (33.3)	5 (20.8)	1.90 (0.27-13.52)	1 (20)	5 (17.9)	1.15 (0.11-12.62)
	[N=6]	*[N=25]*		*[N=5]*	*[N=28]*	
5HTTVNTR (12/12)	3 (50)	9 (36)	1.78 (0.3-10.72)	2 (40)	11 (39.3)	1.03 (0.15-7.19)
HTR1B						
rs6296				3 (60.0)	11 (39.3)	2.18 (0.31-15.29)
rs6298				3 (60.0)	11 (39.3)	2.18 (0.31-15.29)
HTR2A						
rs6313				2 (40.0)	13 (46.4)	0.72 (0.10-5.01)
rs6314				1 (20.0)	6 (21.4)	0.88 (0.082-9.38)
rs1928040				3 (60.0)	20 (74.1)	0.45 (0.06-3.35)
rs6311				2 (40.0)	14 (50.0)	0.67 (0.10-4.62)
HTR3B						
rs1176744				4 (80.0)	11 (39.3)	5.82 (0.57-59.32)
rs3831455				0	(3.6)	-

N, total number of cases; * not in Hardy Weinberg equilibrium

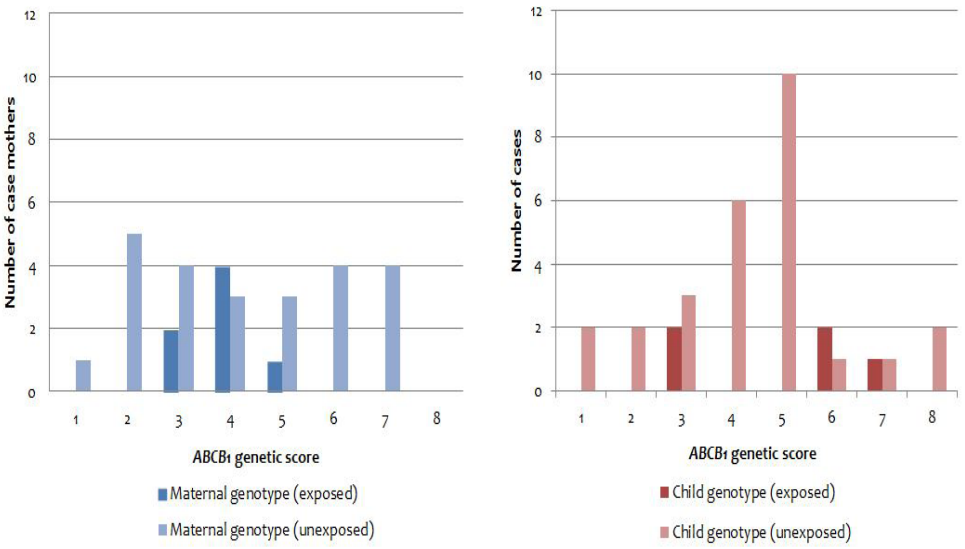

Figure 2: Distribution of maternal and child ABCB1 genetic scoring associated with reduced P-gp function

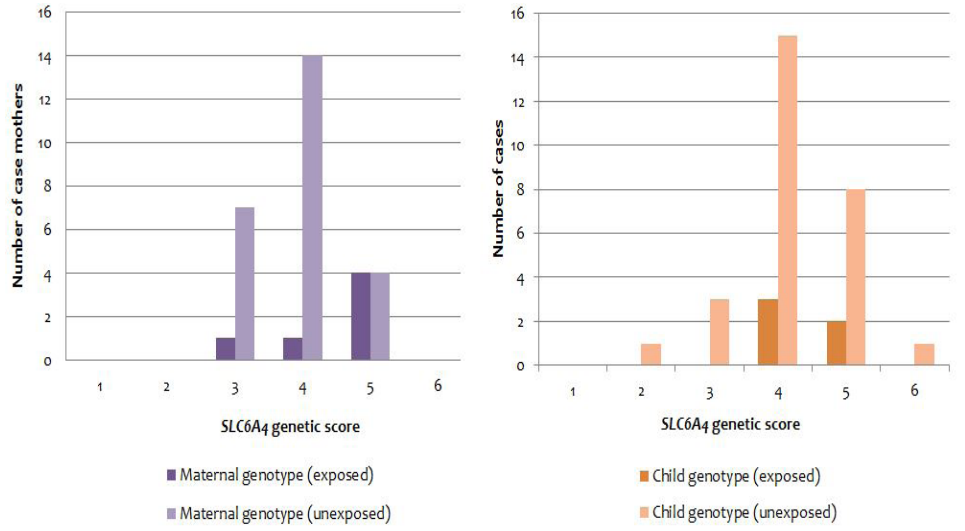

Figure 3: Distribution of maternal and child SLC6A4 genetic scoring associated with increased serotonin transporter function

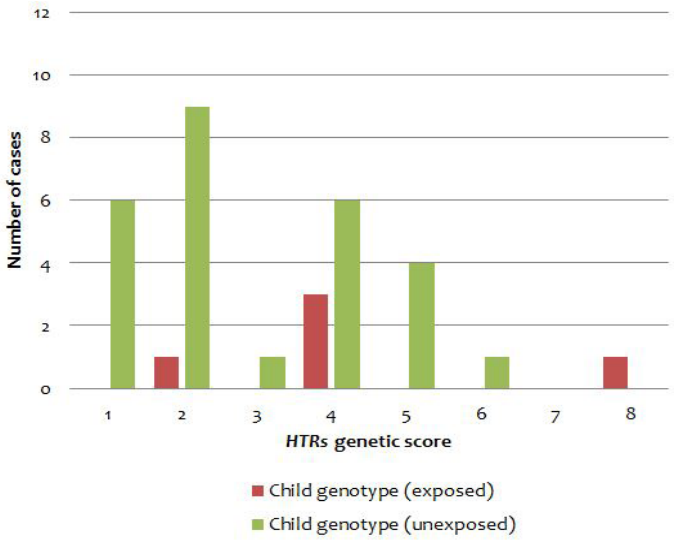

Figure 4: Distribution of child genetic scoring of *HTR* genes associated with increased interaction OR

DISCUSSION

In this exploratory study we tried to find associations of polymorphisms in 10 genes involved in the metabolism of drugs by pregnant women and the occurrence of CHA in their children. Concerning the *ABCB1* SNPs, only maternal rs1128503 had an increased, although non-significant, interaction OR and could therefore be associated with an increased risk of CHA following exposure to SRIs. This SNP, together with rs1045642 (*C3435T*) and rs2030582 (*G2677T/A*), were previously associated with reduced expression/function of placental P-gp and modulated the placental transfer of substrate drugs (29-32). This modulation may affect the protective barrier against xenobiotics in the early stage of pregnancy. It has also been suggested that these SNPs play a role in the clinical response of SRIs because P-gp regulates the transport of SSRIs through the blood-brain barrier (45–50). With regard to congenital anomalies, two previous observational studies reported that maternal and fetal *C3435T* increased fetal susceptibility to CHA and cleft lip following general medication use during pregnancy (39-41). This association was not found in our study, probably because of the different types of medication included in the exposure groups, as we have focused on SRIs use instead of any medication in general.

The L allele of the 5-HTTLPR and 12 repeats of the 5-HTTVNTR of *SLC6A4* had previously been associated with higher efficacy or side effects of SRI treatment, which was proposed to be caused by a higher expression of the SERT (42-46). In this study, the G x E interaction between these variants and SRI-use tended to cause an increase in the risk of CHA, but only for the maternal genotype interaction. Looking at the effect on the fetus, one would expect that the fetal *SLC6A4* variant would have a larger effect on SERT expression in the placenta that is of fetal origin. However, SERT mRNA was also detected in epithelial cells of early decidua, which is the uterine lining of the maternal endometrium (47). The increase in SERT expression may cause a higher response to SRIs, and is manifested by the increased inhibition of 5-HT uptake into the placenta. The exact mechanism seems to be intricate and unclear; however, we can hypothesize that the combination of SERT polymorphisms and SRI-exposure might cause a disruption in the normal 5-HT level available for the transport into the fetal circulation.

Our study found that four SNPs in *HTR* genes encoding for 5-HT receptors showed a possibly increased risk of CHA after the exposure to SRIs, although the effect was not significant. Two of the SNPs, *HTR1A* rs1364043 and *HTR1B* rs6296*,* had previously been associated with an increased response to citalopram (23), while *HTR3B* rs1176744 had been shown to reduce the side effects of paroxetine (48). However, these associations have not yet been replicated in larger studies. On the other hand, *HTR1B* rs6298 was associated with a reduced response to citalopram (23). The role of genetic variations in 5-HT receptors needs further investigation given the importance of 5-HT signaling during embryogenesis, particularly in cell division, differentiation, migration and synaptogenesis (49). Any alteration in the 5-HT level and receptor activity during this period could lead to susceptibility to faulty fetal heart development.

Strengths and limitations

One of the strengths of this study is that it is the first attempt at elucidating the role of pharmacogenetics in the development of CHA associated with prenatal use of SRIs. A further strength is the G x E interaction approach, which is a powerful design for determining the contribution of genetics to adverse drug events or teratogenicity. Previous studies have identified several genetic variations associated with CHA in the presence of environmental factors like maternal obesity, tobacco use and folic acid intake (50-53). A third strength is that the EUROCAT NNL database used in this study records complete information on maternal risk factors (i.e. smoking-, alcohol-, and medication use). Since all cases were selected from the same database, any misclassification of exposure would be non-differential among exposed and

unexposed cases. Finally, we also included cases of terminated pregnancies, which are usually missing from the health surveillance databases.

There are several limitations of this study. First, a case-only study can only measure the risk of the G x E interaction, not the separated risks of G or E. Second, this design is vulnerable to population stratification, although we can assume this effect is minor in this study since the majority of our population is Caucasian (54). Third, we cannot differentiate between different SRIs and doses in the analysis because of the limited number of cases exposed to SRIs and the exploratory nature of the study. The type of SRI might also be a relevant factor, since SRIs can have different pharmacokinetic and pharmacodynamic characteristics.

The use of pharmacogenetics as a tool in personalized drug therapy has been studied before, but the importance of this concept among pregnant patients is now taking the spotlight (55-58). The pharmacogenetic parameters explored in this study are part of a complex interplay between other genetic variants and environmental factors contributing to CHA. Potential gene-gene (G x G) or G x E interaction can occur within the maternal or fetal genotypes and also between maternal and fetal genotypes (59). Based on our current, still limited, knowledge on the pharmacogenetics of SRIs, we need more genetic studies among pregnant patients with depression in order to promote the safest treatment option for both the mothers and their unborn children.

CONCLUSION

Maternal use of SRIs during the first trimester of pregnancy has long been studied for its association with fetal CHA, although the results to date have been conflicting. In this exploratory study, we were not able to find significant genetic variations that may modulate the risk of CHA in fetuses that were exposed to SRIs in the first trimester of pregnancy. Nevertheless, we found that polymorphisms of 5-HT receptors may play a role. Future studies will need a larger number of exposed cases, and possibly incorporate the effect of maternal G x E and fetal G x E contribution to CHA.

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REFERENCES

1. Charlton R, Jordan S, Pierini A, Garne E, Neville A, Hansen A, et al. Selective serotonin reuptake inhibitor prescribing before, during and after pregnancy: a population-based study in six European regions. BJOG. 2014 Oct 28;(DOI 10.1111/1471-0528.13143).

2. Andrade SE, Raebel MA, Brown J, Lane K, Livingston J, Boudreau D, et al. Use of antidepressant medications during pregnancy: a multisite study. Am J Obstet Gynecol. 2008;198(2):194–5.

3. Alwan S, Reefhuis J, Rasmussen SA, Friedman JM. Patterns of antidepressant medication use among pregnant women in a United States population. J Clin Pharmacol. 2011;51(2):264–70.

4. Wurst KE, Poole C, Ephross SA, Olshan AF. First trimester paroxetine use and the prevalence of congenital, specifically cardiac, defects: a meta-analysis of epidemiological studies. Birth defects Res A, Clin Mol Teratol. 2010;88(3):159–70.

5. Grigoriadis S, VonderPorten EH, Mamisashvili L, Roerecke M, Rehm J, Dennis C-L, et al. Antidepressant exposure during pregnancy and congenital malformations: is there an association? A systematic review and meta-analysis of the best evidence. J Clin Psychiatry. 2013 Apr;74(4):e293-308.

6. Myles N, Newall H, Ward H, Large M. Systematic meta-analysis of individual selective serotonin reuptake inhibitor medications and congenital malformations. Aust N Z J Psychiatry. 2013;47(11):1002–12.

7. Wang S, Yang L, Wang L, Gao L, Xu B, Xiong Y. Selective Serotonin Reuptake Inhibitors (SSRIs) and the Risk of Congenital Heart Defects: A Meta-Analysis of Prospective Cohort Studies. J Am Heart Assoc. 2015;4(e001681 doi: 10.1161/JAHA.114.001681)).

8. Daud A, Bergman J, Kerstjens-Frederikse W, Groen H, Wilffert B. The Risk of Congenital Heart Anomalies Following Prenatal Exposure to Serotonin Reuptake Inhibitors—Is Pharmacogenetics the Key? Int J Mol Sci. 2016;17(8):1333; doi:10.3390/ijms17081333.

9. Swen JJ, Wilting I, de Goede AL, Grandia L, Mulder H, Touw DJ, et al. Pharmacogenetics: from bench to byte. Clin Pharmacol Ther. 2008;83(5):781–7.

10. Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, et al. Pharmacogenetics: from bench to byte-an update of guidelines. Clin Pharmacol Ther. 2011;89(5):662–73.

11. Daud AN, Bergman JE, Bakker MK, Wang H, de Walle HEK, Plösch T, et al. Pharmacogenetics of drug-induced birth defects: the role of polymorphisms of placental transporter proteins. Pharmacogenomics. 2014;15(7):1029–41.

12. Fabbri C, Minarini A, Niitsu T, Serretti A. Understanding the pharmacogenetics of selective serotonin reuptake inhibitors. Expert Opin Drug Metab Toxicol. 2014;10(8):1093–118.

13. Kroeze Y, Zhou H, Homberg JR. The genetics of selective serotonin reuptake inhibitors. Pharmacol Ther. 2012 Dec;136(3):375–400.

14. Wilkie MJ, Smith G, Day RK, Matthews K, Smith D, Blackwood D, et al. Polymorphisms in the SLC6A4 and HTR2A genes influence treatment outcome following antidepressant therapy. Pharmacogenomics J. 2009 Feb;9(1):61–70.

15. Hassanzadeh J, Moradzadeh R, Rajaeefard A, Tahmasebi S, Golmohammadi P. A Comparison of Case-control and Case-only Designs to Investigate Gene-environment Interactions using Breast Cancer Data. Iran J

Med Sci. 2012;37(2):112–8.

16. Li D, Conti D V. Detecting gene-environment interactions using a combined case-only and case-control approach. Am J Epidemiol. 2009;169(4):497–504.

17. Albert P, Ratnasinghe D, Tangrea J, Wacholder S. Limitations of the case-only design for identifying gene-environment interactions. Am J Epidemiol. 2001;154(8):687–93.

18. European Surveillance of Congenital Anomalies (EUROCAT). Subgroups of Congenital Anomalies (Version 2012) . http://www.eurocat-network.eu/content/EUROCAT-Guide-1.4-Section-3.3.pdf. Last accessed 10 June 2016.

19. Akamine Y, Yasui-Furukori N, Ieiri I, Uno T. Psychotropic drug-drug interactions involving P-glycoprotein. CNS Drugs. 2012 Nov;26(11):959–73.

20. Hodges LM, Markova SM, Chinn LW, Gow JM, Kroetz DL, Klein TE, et al. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). Pharmacogenet Genomics. 2011 Mar;21(3):152–61.

21. Sadler TW. Selective serotonin reuptake inhibitors (SSRIs) and heart defects: potential mechanisms for the observed associations. Reprod Toxicol. 2011 Dec;32(4):484–9.

22. Gex-Fabry M, Eap CB, Oneda B, Gervasoni N, Aubry J-M, Bondolfi G, et al. CYP2D6 and ABCB1 genetic variability: influence on paroxetine plasma level and therapeutic response. Ther Drug Monit. 2008;30(4):474–82.

23. Villafuerte SM, Vallabhaneni K, Sliwerska E, McMahon FJ, Young EA, Burmeister M. SSRI response in depression may be influenced by SNPs in HTR1B and HTR1A. Psychiatr Genet. 2009 Dec;19(6):281–91.

24. Cariaso M, Lennon G. SNPedia: A wiki supporting personal genome annotation, interpretationand analysis. Nucleic Acids Res. 2012;40:1308–12.

25. Caudle KE, Dunnenberger HM, Freimuth RR, Peterson JF, Burlison JD, Whirl-Carrillo M, et al. Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC). Genet Med. 2016;(January):1–9.

26. Swen JJ, Guchelaar H-JJ, Baak-Pablo RF, Assendelft WJJ, Wessels JAM. Genetic risk factors for type 2 diabetes mellitus and response to sulfonylurea treatment. Pharmacogenet Genomics. 2011;21(8):461–8.

27. Nelveg-Kristensen KE, Madsen MB, Torp-Pedersen C, Køber L, Egfjord M, Rasmussen HB, et al. Pharmacogenetic risk stratification in angiotensin-converting enzyme inhibitor-treated patients with congestive heart failure: A retrospective cohort study. PLoS One. 2015;10(12):e0144195. doi:10.1371/journal.pone.0144195.

28. Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med. 2008;359(21):2208–19.

29. Hitzl M, Schaeffeler E, Hocher B, Slowinski T, Halle H, Eichelbaum M, et al. Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). Pharmacogenetics. 2004;14(5):309–18.

30. Molsa M, Heikkinen T, Hakkola J, Hakala K, Wallerman O, Wadelius M, et al. Functional role of P-glycoprotein in the human blood-placental barrier. Clin Pharmacol Ther. 2005 Aug;78(2):123–31.

31. Rahi M, Heikkinen T, Härtter S, Hakkola J, Hakala K, Wallerman O, et al. Placental transfer of quetiapine in relation to

P-glycoprotein activity. J Psychopharmacol. 2007 Sep;21(7):751–6.

32. Hemauer SJ, Nanovskaya TN, Abdel-Rahman SZ, Patrikeeva SL, Hankins GD V, Ahmed MS. Modulation of human placental P-glycoprotein expression and activity by MDR1 gene polymorphisms. Biochem Pharmacol. 2010 Mar 15;79(6):921–5.

33. Noordam R, Aarts N, Hofman A, van Schaik RHN, Stricker BH, Visser LE. Association between genetic variation in the ABCB1 gene and switching, discontinuation, and dosage of antidepressant therapy: results from the Rotterdam Study. J Clin Psychopharmacol. 2013;33(4):546–50.

34. Kato M, Fukuda T, Serretti A, Wakeno M, Okugawa G, Ikenaga Y, et al. ABCB1 (MDR1) gene polymorphisms are associated with the clinical response to paroxetine in patients with major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2008 Feb;32(2):398–404.

35. Nikisch G, Eap CB, Baumann P. Citalopram enantiomers in plasma and cerebrospinal fluid of ABCB1 genotyped depressive patients and clinical response: a pilot study. Pharmacol Res. 2008;58(5–6):344–7.

36. Lin KM, Chiu YF, Tsai IJ, Chen CH, Shen WW, Liu SC, et al. ABCB1 gene polymorphisms are associated with the severity of major depressive disorder and its response to escitalopram treatment. Pharmacogenet Genomics. 2011 Apr;21(4):163–70.

37. Singh AB, Bousman CA, Ng CH, Byron K, Berk M. ABCB1 polymorphism predicts escitalopram dose needed for remission in major depression. Transl Psychiatry. 2012;2:e198.

38. Fukui N, Suzuki Y, Sawamura K, Sugai T,Watanabe J, Inoue Y, et al. Dose-dependent effects of the 3435 C>T genotype of ABCB1 gene on the steady-state plasma concentration of fluvoxamine in psychiatric patients. Ther Drug Monit. 2007 Apr;29(2):185–9.

39. Obermann-Borst SA, Isaacs A, Younes Z, van Schaik RHN, van der Heiden IP, van Duyn CM, et al. General maternal medication use, folic acid, the MDR1 C3435T polymorphism, and the risk of a child with a congenital heart defect. Am J Obstet Gynecol. 2011 Mar;204(3):236.e1-8.

40. Bliek BJB, van Schaik RHN, van der Heiden IP, Sayed-Tabatabaei FA, van Duijn CM, Steegers EAP, et al. Maternal medication use, carriership of the ABCB1 3435C > T polymorphism and the risk of a child with cleft lip with or without cleft palate. Am J Med Genet A. 2009 Oct;149A(10):2088–92.

41. Wang C, Zhou K, Xie L, Li Y, Zhan Y,Qiao L, et al. Maternal medication use, fetal 3435 C>T polymorphism of the ABCB1 gene, and risk of isolated septal defects in a Han Chinese population. Pediatr Cardiol. 2014;35:1132–41.

42. Kato M, Fukuda T, Wakeno M, Fukuda K, Okugawa G, Ikenaga Y, et al. Effects of the serotonin type 2A, 3A and 3B receptor and the serotonin transporter genes on paroxetine and fluvoxamine efficacy and adverse drug reactions in depressed Japanese patients. Neuropsychobiology. 2006;53:186–95.

43. Lee SH, Choi TK, Lee E, Seok JH, Lee HS, Kim SJ. Serotonin transporter gene polymorphism associated with short-term treatment response to venlafaxine. Neuropsychobiology. 2010 Aug;62(3):198–206.

44. Hu X-Z, Rush AJ, Charney D, WilsonAF, Sorant AJM, Papanicolaou GJ, et al. Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. Arch Gen Psychiatry. 2007 Jul;64(7):783–92.

45. Murphy GM, Hollander SB, Rodrigues

HE, Kremer C, Schatzberg AF. Effects of the serotonin transporter gene promoter polymorphism on mirtazapine and paroxetine efficacy and adverse events in geriatric major depression. Arch Gen Psychiatry. 2004;61(11):1163–9.

46. Staeker J, Leucht S, Laika B, Steimer W. Polymorphisms in serotonergic pathways influence the outcome of antidepressant therapy in psychiatric inpatients. Genet Test Mol Biomarkers. 2014 Jan;18(1):20–31.

47. Bottalico B, Pilka R, Larsson I, Casslen B, Marsal K, Hansson SR. Plasma membrane and vesicular monoamine transporters in normal endometrium and early pregnancy decidua. Mol Hum Reprod. 2003;9(7):389–94.

48. Sugai T, Suzuki Y, Sawamura K, Fukui N, Inoue Y, Someya T. The effect of 5-hydroxytryptamine 3A and 3B receptor genes on nausea induced by paroxetine. Pharmacogenomics J. 2006;6(5):351–6.

49. Bonnin A, Levitt P. Fetal, maternal, and placental sources of serotonin and new implications for developmental programming of the brain. Neuroscience. 2011;197:1–7.

50. Hobbs CA, Cleves MA, Karim MA, Zhao W, MacLeod SL. Maternal folate-related gene environment interactions and congenital heart defects. Obs Gynecol. 2010;116:316–22.

51. Hobbs CA, Cleves MA, Macleod SL, Erickson SW, Tang X, Li J, et al. Conotruncal heart defects and common variants in maternal and fetal genes in folate, homocysteine, and transsulfuration pathways. Birth Defects Res Part A - Clin Mol Teratol. 2014;100(2):116–26.

52. Tang X, Nick TG, Cleves MA, Erickson SW, Li M, Li J, et al. Maternal obesity and tobacco use modify the impact of genetic variants on the occurrence of conotruncal heart defects. PLoS One. 2014;9(10):e108903. doi:10.1371/journal.pone.0108903.

53. Tang X, Hobbs CA, Cleves MA, Erickson SW, Macleod SL, Malik S. Genetic variation affects congenital heart defect susceptibility in offspring exposed to maternal tobacco use. Birth Defects Res Part A - Clin Mol Teratol. 2015;103(10):834–42.

54. Wang LY, Lee WC. Population stratification bias in the case-only study for gene-environment interactions. Am J Epidemiol. 2008;168(2):197–201.

55. Haas DM, D'Alton M. Pharmacogenetics and other reasons why drugs can fail in pregnancy: higher dose or different drug? Obstet Gynecol. 2012 Nov;120(5):1176–9.

56. Haas DM. Obstetric therapeutics-How pharmacogenetics may inform drug therapy for pregnant women in the future. Obstet Gynecol Surv. 2013;68(9):650–4.

57. Quinney SK, Patil AS, Flockhart DA. Is personalized medicine achievable in obstetrics? Semin Perinatol. 2014;38(8):534–40.

58. Dorfman EH, Cheng EY, Hebert MF, Thummel KE, Burke W. Prenatal pharmacogenomics: a promising area for research. Pharmacogenomics J. 2016;(May):1–2.

59. Li M, Li J, Wei C, Lu Q, Tang X, Erickson SW, et al. A Three-Way Interaction among Maternal and Fetal Variants Contributing to Congenital Heart Defects. Ann Hum Genet. 2016;80(1):20–31.

chapter 8

General Discussion

Pregnant women comprise a special group with unmet needs when it comes to drug therapy. It is a big challenge to perform studies related to drug teratogenicity and the safety of drug use during pregnancy, as was mentioned in **Chapter 1**. Ideally, before new drugs are approved for their use on the market, their safety and efficacy profile should also be tested in the pregnant population. Clinical trials should be designed to appropriately capture the time-dependent physiological changes throughout pregnancy, where each patient could serve as their own control (1). This will enable clinicians and patients to make an informed choice of a treatment based on evidence from clinical data. However, clinical trials are not usually done in pregnant women due to ethical and legal reasons. Recently, experts have proposed the involvement of pregnant women in clinical trials, provided that minimization of risk for achieving the objectives of the research was considered (1-5).

At present, the knowledge of human teratogenic risks is still limited. This emphasizes the need for increasing the quality of current study designs, and using complementary study designs (which will be discussed further) to assess the potential risk of drug exposures during pregnancy. Research generates more information continuously, but it is a challenge to select relevant information for clinical decision making. In this chapter, I will discuss our main findings and future perspectives towards personalization of drug therapy during pregnancy, with a focus on the risk of drug teratogenicity.

The role of transporter proteins in fetal drug exposure

Drug interactions mediated by transporter proteins

Drugs taken by pregnant women may be transferred to the fetus. Transporter proteins expressed in the placenta are known to be the gates that facilitate or limit the transport of substrate drugs from the maternal circulation to the fetal side (6-9). In **Chapter 2** and **3**, we used literature data to classify different drugs per specific transporter protein; they can either be substrates, inhibitors, inducers, substrate/inhibitors, or substrate/inducers. About 1 in 10 mothers in our case and reference populations had used drugs which are substrates of P-glycoprotein (P-gp) (**Chapter 2**). Meanwhile, the user rates of drugs which are substrates of other transporters were much lower (for example around 6-8% for multidrug resistance-associated protein 1 (MRP1) and 2-3% for breast cancer resistance protein (BCRP)) (**Chapter 3**). As P-gp is considered to be the most relevant transporter protein in drug transport and elimination, it is studied much more extensively than the other transporters (10).

Fetal drug exposure can be modulated by drug-drug interactions mediated by placental transporters. We used the risk of congenital anomalies as a proxy for fetal drug

exposure as the outcome in our studies. In **Chapter 2**, we identified women who used drugs transported by P-gp, and with possible negative effects on the fetus. We found that the women who took these drugs concurrently with an inhibitor of P-gp, had an increased risk of having children with congenital anomalies. This finding was supported in the literature, mainly in animal and *in vitro/ex vivo* studies which showed that P-gp has a role in limiting fetal exposure to toxicants (10,11). These preclinical studies, however, mainly used placental models obtained in late pregnancy, leaving us with a knowledge gap on the drug transport mechanism during early pregnancy (12-15). The expression of placental P-gp is assumed to be much higher in early pregnancy, as compared to late pregnancy (16,17), suggesting that the placenta's ability to protect the fetus from drugs is greater in early pregnancy. Therefore the effect of the P-gp inhibition on fetal drug transfer might have been more pronounced during this period.

The role of other placental transporters in fetal drug transfer is still understudied. Our study in **Chapter 3** did not find any association between drug interactions mediated by these transporters and the risk of congenital anomalies (i.e. breast cancer resistance protein (BCRP) and multidrug resistance-associated protein 1 (MRP1) as efflux transporters; and organic cation transporter 3 (OCT3), equilibrative nucleoside transporter (ENT1), organic anion transporter 4 (OAT4), organic anion transporting polypeptide 2B1 (OATP2B1) and monocarboxylate transporters 1, 4, 8 and 10 (MCT1, MCT4, MCT8, MCT10) as solute carrier proteins). One major point we learnt from this study is that there is a lack of data with many drugs that are not yet characterized clinically as substrates, inhibitors or inducers of these transporter proteins.

Several transporter-mediated drug interactions have been reported to cause clinical changes on the pharmacokinetics of several substrate drugs. For example, the inhibition of P-gp expressed in the renal tubules and the intestines increases the plasma concentration of digoxin, a P-gp substrate (18). For the OATP uptake transporters expressed in the hepatocytes (OATP1B1, OATP1B3, OATP2B1), co-administration of pravastatin (substrate) and gemfibrozil (inhibitor) leads to an increase in the plasma concentration of pravastatin (19). However, translating these findings for transporters expressed in the placenta can be challenging. Furthermore, previous preclinical and clinical studies investigated only selected substrate drugs. Digoxin, for example, is one of the most extensively used substrate in drug interaction studies mediated by P-gp. Due to narrow therapeutic index of digoxin and its negligible degree of metabolism, differences in the efflux activity of P-gp would have a major impact on its pharmacokinetics (18). However, extrapolating the results to our drugs of interest, which could have different physicochemical properties,

needed further evidence. It is also a big challenge to measure the changes in placental transport of drugs at the relevant concentration expected in patients. Furthermore, there are other parameters contributing to the levels of fetal drug exposure, including the metabolism of drugs by metabolic enzymes, drug interactions mediated by these enzymes, and pharmacokinetic changes of a drug throughout pregnancy (20). Also, large confidence intervals were found in our studies, pointing to a need for larger studies to replicate the findings and improve the clinical relevance.

Genetic polymorphisms of transporter proteins

Besides the effect of drug inhibition of the placental transporters, the expression and activity of a transporter can also be modulated by genetic polymorphisms. In **Chapter 4** we reviewed all genetic polymorphisms that were reported to be related to the expression and transport activity of placental transporter proteins. We also proposed to group these relevant genetic variants into phenotype classifications, as previously used for metabolic enzymes (21). These phenotypes are based on their effect on transporter function in the placenta: 'increased', 'normal', 'decreased' and 'abolished' activities. Similar to the genetic risk scoring method, every risk allele counted is given the same absolute effect size on the phenotype scale, either as increased, normal, decreased or abolished transport (22,23). Another assumption is that the same phenotype applies to all possible substrates of the same transporter. The limitation of these assumptions is that it may not be a true reflection of the biological basis of transporter activity.

The usefulness of this phenotype scoring is yet to be demonstrated in a clinical setting. By using this phenotype grouping, it is possible to perform genetic association studies using smaller and more realistic sample sizes. Such studies might further explain the associations previously found between P-gp rs1045642 (*3435C>T*), maternal drug/toxicant exposures, and the risk of congenital anomalies (24-26). Taking into account the non-genetic factors, especially maternal drug use, gene-environment interaction studies are one of the options in investigating the safety of drugs used during the first trimester. Two common polymorphisms, OATP1B1 rs4149056 (*521T>C*) and BCRP rs2231142 (*421C>A*), have also been reported to have sufficient impact on medication disposition or response, which warrant their incorporation into the drug development process (27,28).

In general, our studies point out the relevance of P-gp as one of the protective mechanisms against the possible harmful effect of a drug on the developing fetus. However, it is unknown whether the role of P-gp mediated drug-drug interactions is as large as the role of the drug-drug interactions mediated by metabolic enzymes,

which are currently being optimized for the implementation in clinical decision supports (29,30). As outlined by The International Transporter Consortium, future studies on drug transporters could start from *in vitro* studies to determine whether the drug of interest is a substrate to any of the placental transporters (27). If drug-drug interactions mediated by these transporters contribute significantly to the drug's pharmacokinetics, and if the drug has a small therapeutic window, pharmacogenomic studies should then be considered. A better understanding of the ontogeny in the expression of placental transporter proteins is also of clinical importance. To facilitate future research, the established public data repositories in drug transporter studies can be useful in knowledge sharing, e.g. the UCSF–FDA Transportal21 (http://bts.ucsf.edu/fdatransportal) and PharmGKB (http://www.pharmgkb.org) (31).

Pharmacogenetics as a tool towards personalized drug therapy

Pharmacogenetics can be a tool to identify patients who require changes in drug dosing or selection, in order to ensure the efficacy and safety of the drug therapy. Before investing in the implementation of this concept on a national scale, we need to educate the public on pharmacogenetics. Surveys assessing the knowledge and attitude towards pharmacogenetics, in the early 2000's, were targeted to specific patient populations or racial groups (32,33). More recent studies have extended the focus towards the public and health care providers, and new insights of the challenges were gained from both population groups.

As our focus group is pregnant women, we assessed the knowledge and attitude of the concept of pharmacogenetics among women who recently became mothers (**Chapter 5**). We observed that many of the respondents (nearly 70%, N=219) are aware of the relation between their genes and the response of their body to medication, although only few of them knew the term 'pharmacogenetics'. The use of this term might also be one of the reasons of the low response rate (22%) in our study, as it might be intimidating and might have caused the women to refrain from participating.

Our respondents were generally positive towards the implementation of pharmacogenetics in clinical care. However, some concerns were raised, which were mostly consistent with the ones reported in the literature. They included the privacy and anonymity of genetic information, possible misuse by employers or insurance companies and a lack of understanding of the concept (32,34-37). Our survey also found that the public expects to receive relevant information from their health care providers, while according to other surveys, the health care providers were concerned about their lack of knowledge of pharmacogenetics (38,39). Other

barriers perceived by the health care providers were the cost and reimbursement for the service, shortage of personnel, lack of clinical guidelines and time constraints (38-40). The gap between patients' high expectation of information and health care providers' limited knowledge highlights the need for better knowledge dissemination on pharmacogenetics. This calls for a more uniform educational program and training for the pharmacists and medical doctors in interpreting pharmacogenetic information and translating it into clinical care (41,42).

For future studies, it is important to improve the participation rates. When conducting surveys involving patient populations or the public, the choice of terms used might be important. Other terms within the context of pharmacogenetics include 'personalized medicine/drug therapy', 'precision medicine', 'individualized medicine', which are easier and more likely to be used in the general media. In addition, more effort should be given to educate the public on the concept of pharmacogenetics and what it can offer towards better drug therapy options, for example through seminars, brochures, websites or using social media platforms.

Pharmacogenetics and drug-induced teratogenicity

As an effort to pave the way to personalized drug therapy during pregnancy, we explored the use of pharmacogenetic research in determining fetal risk of teratogenicity. Finding pharmacogenetic markers relevant for the risk of congenital anomalies can be a tool in preventive measures against drug teratogenicity. We explored the use of this tool in assessing the risk of congenital heart anomalies (CHA) associated with the use of serotonin reuptake inhibitors (SRIs) in the first trimester of pregnancy. We first identified possible pharmacogenetic predictors in relation to the pharmacokinetics of SRIs and the proposed teratogenic effect on the fetal heart (**Chapter 6**). Important components in the mechanism of this purported teratogenicity include the maternal metabolic enzymes, placental transporter proteins, serotonin transporter and serotonin receptors. Although the human placenta and the developing fetus each have some minor metabolizing capacity, both seem unlikely to contribute to the total pharmacokinetics of drugs taken by the mother (43). The dominant metabolic enzyme in the fetal liver is CYP3A7, which participates in the synthesis of estrogens. However, the interindividual variability of CYP3A7 expression was high and data on its role in the metabolism of drugs was scarce (44,45).

In **Chapter 7**, we performed an exploratory gene-environment (G x E) interaction study to explore the G x E effect of the pharmacogenetic predictors and prenatal exposure to SRIs on the risk of CHA. Among several single nucleotide polymorphisms (SNPs) tested, fetal genotypes in serotonin receptors (*HTR1A*

rs1364043, *HTR1B* rs6296 & rs6298, *HTR3B* rs1176744) seemed to interact with SRI exposure to cause an increased risk of CHA. However, we had too limited sample sizes for these associations to reach statistical significance. The participation rate was low (30% among cases exposed to SRIs), despite our efforts of sending reminders and collecting DNA via buccal swabs instead of blood collection. We also offered them the results of their pharmacogenetic tests relevant for the dosing of certain drugs, which are quite costly. We understand that we are dealing with a rather difficult population, as congenital anomalies can be a significant medical and psychological burden to the families affected. In addition, the parents may not understand the benefit of such pharmacogenetic tests for themselves. To improve participation rate, it would be helpful to first educate them on the pertinent role of genetics in understanding the etiology and risk of congenital anomalies. Ways to approach them can be extended through the physician or specialist who treats the child, relevant support groups, or organizations that are directly involved in the well-being and support for these families.

Our exploratory study faced several challenges, which contribute to some limitations. CHA can range from very mild (unnoticed, undiagnosed) to very severe (leading to spontaneous abortion or termination of pregnancy). The range in severity creates a selection bias towards cases with more severe CHA which were detected within a few years of life. Also, G x E studies are prone to inadvertent bias in the selection of candidate genes, and there is the risk of finding false positive associations as a result from multiple testing (46). Therefore, it is crucial to replicate the findings with a large enough sample to identify the initial association. In general, sample sizes required to detect the G x E interaction are larger than those required to detect main genetic or environmental effects. For this, collaborations between registries of congenital anomalies are needed.

There are other aspects that we can improve in this study. First, it is important for future research to consider the effect of gene-gene interactions when identifying the cumulative effect of genetic variations on the abnormal phenotype (46). As an example, genes associated with folate, homocysteine and transsulfuration pathways (methylene tetrahydrofolate reductase (MTHFR) and thymidylate synthetase (TYMS)) are important in modulating plasma folic acid concentration in the fetus, which is also a factor in the development of CHA (47,48). Second, genetic predispositions from the paternal side may also be part of the complex etiology of drug teratogenicity, next to the effects of both the mother and the fetus (49). It might be useful to perform a case-parent triad design, which also includes the fathers as participants, to gain insight in the role of paternal genetic variants. Finally, it is important to acknowledge that multiple interaction effects may occur between maternal exposure to drugs with either

the mother's genes or the infant's genes, resulting in maternal G x E and infant G x E effects (50). Many considerations need to be taken into account in G x E studies of congenital anomalies highlighting the importance of a proper study design and prior knowledge of which type of genetic information to collect and which statistical approaches to use.

Future perspectives

The future of pharmacogenetics in drug therapy in pregnancy can be foreseen in terms of its clinical application in both future patient care and research possibilities. In future patient care, pregnant women may benefit from personalized drug therapy, which will deliver tailored drug dosing and selection to ensure drug efficacy with limited toxicities. This will enable medical practitioners to counsel on antenatal drug selection by recognizing those fetuses who are at a higher risk for drug teratogenicity. In research, identifying which children were susceptible for drug toxicity based on their pharmacogenetic risk factors may elucidate the mechanism involved in drug teratology (43).

The initial step towards personalized drug therapy for pregnant women is identifying important genetic variants, or 'pharmacogenetic predictors', associated with drug-induced teratogenicity. More pharmacogenetic studies are needed to enrich the existing knowledge, and they need to be replicated (**Box 1**). The pharmacogenetic predictors relevant for drug pharmacokinetics or pharmacodynamics shall be incorporated as a parameter in drug modeling approaches, e.g. pharmacokinetic/pharmacodynamic (PK/PD) and physiologically-based pharmacokinetic (PBPK) models in pregnancy (51-54). These models are being developed as a quantitative prediction model for drug pharmacokinetics and dosing in pregnant women, which may include fetal and/or amniotic compartments to better characterize the placental drug transfer (55).These models will then need validation before implementing it in clinical recommendations.

Although pharmacogenetics is gaining attention, the implementation of this knowledge in drug therapy is truly challenging. Many of the results from association studies are contradictory, or are performed in a small patient population that might not be homogenous in terms of drug treatment. As we strive to prevent drug-induced teratogenicity, we could expect more options in the scope of non-invasive prenatal genetic screening. This screening is now focused on the detection of fetal chromosomal abnormalities (aneuploidy), using cell-free fetal DNA in the maternal circulation (56,57). The use of advanced molecular tools, including digital polymerase chain reaction and SNP genotyping microarray, has also increased the possibility of

a complete non-invasive fetal genotyping in utero (56). It would be a big challenge to run genetic tests in unborn babies as a routine clinical procedure, but it should be an option, at least for those with other known risk factors.

With the rapid growth of genomic analysis technology and continuously decreasing costs of genotyping, the ability to predict a fetus's risk of teratogenicity may be achievable in the future. However, provided that we are able to detect fetal phenotypes associated with an increased risk of drug-induced teratogenicity, are we able to implement this knowledge in a preventive manner? Pharmacogenetic screening may be able to select high-risk subjects that may benefit from dose changes or alternative drugs. However, the contribution of pharmacogenetic predictors may explain only parts of the total prediction risk. Therefore, we need more data to establish a treatment plan aimed to provide an efficacious treatment to the mother with the lowest possible risk to the fetus.

Putting the context of this thesis into clinical practice seems quite distant, but it could be one step towards understanding the mechanistic pathway of the teratogenicity of a drug taken during pregnancy. Therefore, we need continuous research in the areas of epidemiology and genetics, as well as epigenetics, to shed light on the risk factors of teratogenicity, which will hopefully be valuable in preventive strategies in clinical practice.

Box 1: Alternative approaches in genetic studies related to drug-induced teratogenicity

- Genome-wide association studies (GWAS) have been useful in detecting common variants associated with congenital anomalies, especially CHA, cleft lip and/or palate (CLP) and hypospadias (49). These genetic risk variants could then be included in G x E as one of the predictors relevant in the development of drug teratogenicity.

- The G x E wide interaction studies (GEWIS), an extension from GWAS, is the approach of detecting G x E effects from the signals obtained from GWAS. It begins with the identification of SNPs which are associated with the outcome, and then evaluates the SNPs for their interactions with environmental exposures with the traditional case-control G x E studies (58,59).

- Epigenetic modifications, especially DNA methylation, were also found to be an underlying mechanism in the development of CHA (60,61). Various maternal factors implicated with abnormal fetal development have been shown to affect DNA methylation patterns. The combined effects of pharmacogenetics and epigenetics on fetal development are yet to be explored.

REFERENCES

1. Gonzalez D, Boggess KA, Cohen-Wolkowiez M. Lessons Learned in Pediatric Clinical Research to Evaluate Safe and Effective Use of Drugs in Pregnancy. Obstet Gynecol. 2015;125(4):953–8.

2. Briggs GG, Polifka JE, Wisner KL, Gervais E, Miller RK, Berard A, et al. Should pregnant women be included in phase IV clinical drug trials? Am J Obstet Gynecol. 2015;213(6):810–5.

3. Lyerly AD, Little MO, Faden RR. Reframing the Framework: Toward Fair Inclusion of Pregnant Women as Participants in Research. Am J Bioeth. 2011;11(5):50–68.

4. Coverdale JH, McCullough LB, Chervenak FA. The ethics of randomized placebo-controlled trials of antidepressants with pregnant women: a systematic review. Obstet Gynecol. 2008;112(6):1361–8.

5. Chervenak FA, McCullough LB. An ethically justified framework for clinical investigation to benefit pregnant and fetal patients. Am J Bioeth. 2011;11(5):39–49.

6. Vahakangas K, Myllynen P. Drug transporters in the human blood-placental barrier. Br J Pharmacol. 2009;158(3):665–78.

7. Iqbal M, Audette MC, Petropoulos S, Gibb W, Matthews SG. Placental drug transporters and their role in fetal protection. Placenta. 2012;33(3):137–42.

8. Staud F, Cerveny L, Ceckova M. Pharmacotherapy in pregnancy; effect of ABC and SLC transporters on drug transport across the placenta and fetal drug exposure. J Drug Target. 2012 Nov;20(9):736–63.

9. Bloise E, Ortiga-Carvalho TM, Reis FM, Lye SJ, Gibb W, Matthews SG. ATP-binding cassette transporters in reproduction: A new frontier. Hum Reprod Update. 2016;22(2):164–81.

10. Ceckova-Novotna M, Pavek P, Staud F. P-glycoprotein in the placenta: expression, localization, regulation and function. Reprod Toxicol. 2006;22(3):400–10.

11. Prouillac C, Lecoeur S. The role of the placenta in fetal exposure to xenobiotics: importance of membrane transporters and human models for transfer studies. Drug Metab Dispos. 2010;38(10):1623–35.

12. Molsa M, Heikkinen T, Hakkola J, Hakala K, Wallerman O, Wadelius M, et al. Functional role of P-glycoprotein in the human blood-placental barrier. Clin Pharmacol Ther. 2005 Aug;78(2):123–31.

13. Ceccaldi P-F, Gavard L, Mandelbrot L, Rey E, Farinotti R, Treluyer J-M, et al. Functional role of p-glycoprotein and binding protein effect on the placental transfer of lopinavir/ritonavir in the ex vivo human perfusion model. Obstet Gynecol Int. 2009 Jan;doi 10.115:1–6.

14. Hemauer SJ, Patrikeeva SL, Nanovskaya TN, Hankins GD V, Ahmed MS. Opiates inhibit paclitaxel uptake by P-glycoprotein in preparations of human placental inside-out vesicles. Biochem Pharmacol. 2009 Nov 1;78(9):1272–8.

15. Hemauer SJ, Patrikeeva SL, Nanovskaya TN, Hankins GD V, Ahmed MS. Role of human placental apical membrane transporters in the efflux of glyburide, rosiglitazone, and metformin. Am J Obstet Gynecol. 2010 Apr;202(4):383.e1-7.

16. Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, Gibb W. Expression of the Multidrug Resistance P-Glycoprotein, (ABCB1 glycoprotein) in the Human Placenta Decreases with Advancing Gestation. Placenta. 2006;27(6–7):602–9.

17. Gil S, Saura R, Forestier F, Farinotti R. P-glycoprotein expression of the human

placenta during pregnancy. Placenta. 2005;26(2–3):268–70.

18. Muller F, Fromm MF. Transporter-mediated drug–drug interactions. Pharmacogenomics. 2011;12(7):1017–37.

19. Kyrklund C, Backman JT, Neuvonen M, Neuvonen PJ. Gemfibrozil increases plasma pravastatin concentrations and reduces pravastatin renal clearance. Clin Pharmacol Ther. 2003;73(6):538–44.

20. Isoherranen N, Thummel KE. Drug metabolism and transport during pregnancy : How does drug disposition change during pregnancy and what are the mechanisms that cause such changes ? Drug Metab Dispos. 2013;41:256–62.

21. Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, et al. Pharmacogenetics: from bench to byte-an update of guidelines. Clin Pharmacol Ther. 2011;89(5):662–73.

22. Janssens ACJ, van Duijn CM. Genome-based prediction of common diseases: methodological considerations for future research. Genome Med. 2009;1(2):20.

23. Dudbridge F. Power and predictive accuracy of polygenic risk scores. PLoS Genet. 2013;9(3):e1003348.

24. Wang C, Xie L, Zhou K, Zhan Y, Li Y, Li H, et al. Increased risk for congenital heart defects in children carrying the ABCB1 Gene C3435T polymorphism and maternal periconceptional toxicants exposure. PLoS One. 2013 Jan;8(7):e68807.

25. Obermann-Borst SA, Isaacs A, Younes Z, van Schaik RHN, van der Heiden IP, van Duyn CM, et al. General maternal medication use, folic acid, the MDR1 C3435T polymorphism, and the risk of a child with a congenital heart defect. Am J Obstet Gynecol. 2011 Mar;204(3):236.e1-8.

26. Bliek BJB, van Schaik RHN, van der Heiden IP, Sayed-Tabatabaei FA, van Duijn CM, Steegers EAP, et al. Maternal medication use, carriership of the ABCB1 3435C > T polymorphism and the risk of a child with cleft lip with or without cleft palate. Am J Med Genet A. 2009 Oct;149A(10):2088–92.

27. Giacomini KM, Balimane P V, Cho SK, Eadon M, Edeki T, Hillgren KM, et al. International Transporter Consortium commentary on clinically important transporter polymorphisms. Clin Pharmacol Ther. 2013;94(1):23–6.

28. Giacomini KM, Huang S-M, Tweedie DJ, Benet LZ, Brouwer KLR, Chu X, et al. Membrane transporters in drug development. Nat Rev Drug Discov. 2010;9(3):215–36.

29. Scheife RT, Hines LE, Boyce RD, Chung SP, Momper J, Sommer CD, et al. Consensus Recommendations for Systematic Evaluation of Drug-Drug Interaction Evidence for Clinical Decision Support Richard. Drug Saf. 2015;38(2):197–206.

30. Tilson H, Hines LE, McEvoy G, Weinstein DM, Hansten PD, Matuszewski K, et al. Recommendations for selecting drug-drug interactions for clinical decision support. Am J Heal Pharm. 2016;73(8):395–401.

31. Giacomini KM, Huang S-M. Transporters in drug development and clinical pharmacology. Clin Pharmacol Ther. 2013;94(1):3–9.

32. Bevan JL, Lynch JA, Dubriwny TN, Harris TM, Achter PJ, Reeder AL, et al. Informed lay preferences for delivery of racially varied pharmacogenomics. Genet Med. 2003;5(5):393–9.

33. Rogausch A, Prause D, Schallenberg A, Brockmöller J, Himmel W. Patients' and physicians' perspectives on pharmacogenetic testing. Pharmacogenomics. 2006;7(1):49–59.

34. Haddy CA, Ward HM, Angley MT, McKinnon RA. Consumers' views of

pharmacogenetics--A qualitative study. Res Social Adm Pharm. 2010 Sep;6(3):221–31.

35. O'Daniel J, Lucas J, Deverka P, Ermentrout D, Silvey G, Lobach DF, et al. Factors influencing uptake of pharmacogenetic testing in a diverse patient population. Public Health Genomics. 2009;13(1):48–54.

36. Kobayashi E, Sakurada T, Ueda S, Satoh N. Public involvement in pharmacogenomics research: A national survey on patients' attitudes towards pharmacogenomics research and the willingness to donate DNA samples to a DNA bank in Japan. Cell Tissue Bank. 2011;12:71–80.

37. Shah J. Criteria influencing the clinical uptake of pharmacogenomic strategies. BMJ Br Med J. 2004;328(7454):1482–6.

38. Alexander KM, Divine HS, Hanna CR, Gokun Y, Freeman PR. Implementation of personalized medicine services in community pharmacies : Perceptions of independent community pharmacists. J Am Pharm Assoc Pract Pharm Ed. 2014;54(5):510–7.

39. Elewa H, Alkhiyami D, Alsahan D, Abdel-Aziz A. A survey on the awareness and attitude of pharmacists and doctors towards the application of pharmacogenomics and its challenges in Qatar. J Eval Clin Pract. 2015;21(4):703–9.

40. Haga S, Burke W, Ginsburg G, Mills R, Agans R. Primary care physicians' knowledge of and experience with pharmacogenetic testing. Clin Genet. 2012;82(4):388–94.

41. Kuo GM, Lee KC, Ma JD. Implementation and outcomes of a live continuing education program on pharmacogenomics. Pharmacogenomics. 2013;14(8):885–95.

42. Pisanu C, Tsermpini E-E, Mavroidi E, Katsila T, Patrinos GP, Squassina A. Assessment of the pharmacogenomics educational environment in Southeast Europe. Public Health Genomics. 2014;17(5–6):272–9.

43. Dorfman EH, Cheng EY, Hebert MF, Thummel KE, Burke W. Prenatal pharmacogenomics: a promising area for research. Pharmacogenomics J. 2016;(May):1–2.

44. Fanni D, Fanos V, Ambu R, Lai F, Gerosa C, Pampaloni P, et al. Overlapping between CYP3A4 and CYP3A7 expression in the fetal human liver during development. J Matern Neonatal Med. 2015;28(11):1291–1295.DOI: 10.3109/14767058.2014.951625.

45. Leeder JS, Gaedigk R, Marcucci K a, Gaedigk A, Vyhlidal C a, Schindel BP, et al. Variability of CYP3A7 Expression in Human Fetal Liver. Pharmacol Exp Ther. 2005;314(2):626–35.

46. Zhu H, Kartiko S, Finnell RH. Importance of gene - environment interactions in the etiology of selected birth defects. Clin Genet. 2009;75(5):409–23.

47. Hobbs CA, Cleves MA, Macleod SL, Erickson SW, Tang X, Li J, et al. Conotruncal heart defects and common variants in maternal and fetal genes in folate, homocysteine, and transsulfuration pathways. Birth Defects Res Part A - Clin Mol Teratol. 2014;100(2):116–26.

48. Xuan C, Li H, Zhao J-X, Wang H-W, Wang Y, Ning C-P, et al. Association Between MTHFR Polymorphisms and Congenital Heart Disease: A Meta-analysis based on 9,329 cases and 15,076 controls. Sci Rep. 2014;4:7311.

49. Webber DM, Macleod SL, Bamshad MJ, Shaw GM, Finnell RH, Shete SS, et al. Developments in our understanding of the genetic basis of birth defects. Birth Defects Res Part A - Clin Mol Teratol. 2015;103(8):680–91.

50. Tai CG, Graff RE, Liu J, Passarelli MN, Mefford JA, Shaw GM, et al. Detecting gene-environment interactions in human

birth defects: Study designs and statistical methods. Birth Defects Res Part A - Clin Mol Teratol. 2015;103(8):692–702.

51. Abduljalil K, Furness P, Johnson TN, Rostami-Hodjegan A, Soltani H. Anatomical, physiological and metabolic changes with gestational age during normal pregnancy. Clin Pharmacokinet. 2012;51(6):365–96.

52. Gaohua L, Abduljalil K, Jamei M, Johnson TN, Rostami-Hodjegan A. A pregnancy physiologically based pharmacokinetic (p-PBPK) model for disposition of drugs metabolized by CYP1A2, CYP2D6 and CYP3A4. Br J Clin Pharmacol. 2012;74(5):873–85.

53. Ke AB, Nallani SC, Zhao P, Rostami-Hodjegan A, Unadkat JD. Expansion of a PBPK model to predict disposition in pregnant women of drugs cleared via multiple CYP enzymes, including CYP2B6, CYP2C9 and CYP2C19. Br J Clin Pharmacol. 2013;77(3):554–70.

54. Xia B, Heimbach T, Gollen R, Nanavati C, He H. A simplified PBPK modeling approach for prediction of pharmacokinetics of four primarily renally excreted and CYP3A metabolized compounds during pregnancy. AAPS J. 2013;15(4):1012–24.

55. Benaboud S, Tréluyer JM, Urien S, Blanche S, Bouazza N, Chappuy H, et al. Pregnancy-related effects on lamivudine pharmacokinetics in a population study with 228 women. Antimicrob Agents Chemother. 2012;56(2):776–82.

56. Liao GJW, Chan KCA, Jiang P, Sun H, Leung TY, Chiu RWK, et al. Noninvasive Prenatal Diagnosis of Fetal Trisomy 21 by Allelic Ratio Analysis Using Targeted Massively Parallel Sequencing of Maternal Plasma DNA. PLoS One. 2012;7(5):e38154.

57. Puszyk WM, Crea F, Old RW. Noninvasive prenatal diagnosis of aneuploidy using cell-free nucleic acids in maternal blood : promises and unanswered questions. Prenat Diagn. 2008;28:1–6.

58. Murcray CE, Lewinger JP, Gauderman WJ. Gene-environment interaction in genome-wide association studies. Am J Epidemiol. 2009;169(2):219–26.

59. Khoury MJ, Wacholder S. Invited Commentary: From Genome-Wide Association Studies to Gene-Environment-Wide Interaction Studies--Challenges and Opportunities. Am J Epidemiol. 2008;169(2):227–30.

60. Chowdhury S, Erickson SW, MacLeod SL, Cleves MA, Hu P, Karim MA, et al. Maternal genome-wide DNA methylation patterns and congenital heart defects. PLoS One. 2011;6(1):e16506.

61. Chowdhury S, Cleves MA, MacLeod SL, James SJ, Zhao W, Hobbs CA. Maternal DNA hypomethylation and congenital heart defects. Birth Defects Res Part A - Clin Mol Teratol. 2011;91(2):69–76.

ADDENDUM

- Appendices
- Summary
- Samenvatting (summary in Dutch)
- Acknowledgement
- List of publications
- Research Institute SHARE
- About the author

APPENDICES FOR CHAPTER 2

Appendix 1.1 (a): Drugs associated with P-gp transport and the number of users in case group and the reference population.

Drugs	Number of users	
	Cases (N=4,634)	Reference population (N=25,126)
P-gp substrates		
Drugs for acid related disorders, A02		
Cimetidine, A02BA01	2	20
Ranitidine, A02BA02	6	142
Drugs for functional gastrointestinal disorders, A03		
Domperidone, A03FA03	8	182
Antidiarrheals, intestinal anti-inflammatory/anti-infective agents, A07		
Loperamide, A07DA03 (OTC)	5	40
Beta-blocking agents, C07		
Propranolol, C07AA05	11	92
Drugs acting on the renin-angiotensin system, C09		
Losartan, C09CA01	0	4
Corticosteroids for systemic use, H02		
Betamethasone, H02AB01	1	7
Methylprednisolone, H02AB04	0	4
Hydrocortisone, H02AB09	3	25
Triamcinolone, H02AB08	7	34
Antibacterials for systemic use, J01		
Doxycycline, J01AA02	102	566
Tetracycline, J01AA07	4	27
Levofloxacin, J01MA12	0	5
Analgesics, N02		
Paracetamol, N02BE01 (OTC)	361	458
Sumatriptan, N02CC01	18	180
Antiepileptics, N03		
Phenobarbital, N03AA02	0	2
Lamotrigine, N03AX09	3	17
Topiramate, N03AX11	0	2
Gabapentin, N03AX12	0	4
Levetiracetam, N03AX14	1	4
Psycholeptics, N05		
Levomepromazine, N05AA02	0	1
Perphenazine, N05AB03	0	1
Clozapine, N05AH02	0	1
Olanzapine, N05AH03	0	21
Risperidone, N05AX08	2	14
Aripiprazole, N05AX12	0	3

Psychoanaleptics, N06		
Imipramine, N06AA02	0	4
Clomipramine, N06AA04	6	42
Trimipramine, N06AA06	0	1
Nortriptyline, N06AA10	2	4
Citalopram, N06AB04	17	101
Escitalopram, N06AB10	0	9
Venlafaxine, N06AX16	8	74
Antihistamines for systemic use, R06		
Fexofenadine, R06AX26	5	81
Cetirizine, R06AE07 (OTC)	15	221
P-gp inhibitor		
Antithrombotic agents, B01		
Dipyridamole, B01AC07	0	5
Sex hormones and modulators of the genital system, G03		
Progesterone, G03DA04	58	273
Psychoanaleptics, N06		
Maprotiline, N06AA21	0	2
Duloxetine, N06AX21	1	4
Antiprotozoals, P01		
Mefloquine, P01BC02	1	5
P-gp substrate/inhibitor		
Drugs for acid related disorders, A02		
Omeprazole, A02BC01	41	295
Pantoprazole, A02BC02	12	36
Lansoprazole, A02BC03	0	6
Diuretics, C03		
Spironolactone, C03DA01	0	1
Lipid modifying agents, C10		
Simvastatin, C10AA01	2	18
Atorvastatin, C10AA05	2	12
Antibacterials for systemic use, J01		
Clarithromycin, J01FA09	30	125
Azithromycin, J01FA10	16	161
Antimycotics for systemic use, J02		
Ketoconazole, J02AB02	1	5
Itraconazole, J02AC02	6	56
Immunosuppressants, L04		
Cyclosporine A, L04AD01	1	4
Psycholeptics, N05		
Fluphenazine, N05AB02	0	1
Haloperidol, N05AD01	4	25
Quetiapine, N05AH04	3	21

Psychoanaleptics, N06		
Amitriptyline, N06AA09	8	101
Fluoxetine, N06AB03	17	110
Paroxetine, N06AB05	44	294
Sertraline, N06AB06	5	35
Fluvoxamine, N06AB08	3	55
Antihistamines for systemic use, R06		
Terfenadine, R06AX12	7	67
P-gp substrate/inhibitor/inducer		
Calcium channel blockers, C08		
Verapamil, C08DA01	0	9
Diltiazem, C08DB01	1	2
Antibacterials for systemic use, J01		
Erythromycin, J01FA01	13	80
Antivirals for systemic use, J05		
Nelfinavir, J05AE04	0	1
Lopinavir, J05AR10 (with ritonavir)	0	1
Other gynecologicals, G02		
Bromocriptine, G02CB01	3	0
Immunosuppressants, L04		
Tacrolimus, L04AD02	0	3
P-gp inducer		
Drugs used in diabetes, A10		
Insulins and analogues, A10A	28	110
Calcium channel blockers, C08		
Nifedipine, C08CA05	0	12
Antineoplastic agents, L01		
Fluorouracil, L01BC02	0	3
P-gp substrate/inducer		
Antimycobacterials, J04		
Rifampicin, J04AB02	0	2
Corticosteroids for systemic use, H02		
Dexamethasone, H02AB02	1	11
Analgesics, N02		
Morphine, N02AA01	1	3
Antiepileptics, N03		
Phenytoin, N03AB02	1	2
Carbamazepine, N03AF01	8	56
P-gp inducer/inhibitor		
Psycholeptics, N05		
Midazolam, N05CD08	2	18

Appendix 1.1 (b): Drugs associated with P-gp transport with no users in both case group and the reference population.

P-gp substrates	P-gp inhibitor	P-gp substrate/inhibitor
Antithrombotic agents, B01 Clopidogrel, B01AC04 *Cardiac therapy agents, C01* Digoxin, C01AA05 *Beta-blocking agents, C07* Talinolol, C07AB13 Celiprolol, C07AB08 *Lipid modifying agents, C10* Lovastatin, C10AA02 *Corticosteroids for systemic use, H02* Aldosterone, H02AA01 *Antibacterials for systemic use, J01* Sparfloxacin, J01MA09 *Antineoplastic agents, L01* Melphalan, L01AA03 Paclitaxel, L01CD01 Docetaxel, L01CD02 Epirubicin, L01DB03 Mitomycin C, L01DC03 Teniposide, L01CB02 Imatinib, L01XE01 Topotecan, L01XX17 Irinotecan, L01XX19 Actinomycin D, L01DA01 *Muscle relaxants, M03* Vecuronium, M03AC03 *Psycholeptics, N05* Bromperidol, N05AD06 *Psychoanaleptics, N06* Desvenlafaxine, N06AX23 *Antihelmintics, P02* Ivermectin, P02CF01	*Antineoplastic agents, L01* Gefitinib, L01XE02 Erlotinib, L01XE03 *Cardiac therapy agents, C01* Propafenone, C01BC03 Ranolazine, C01EB18 Dronedarone, C01BD07 *Beta-blocking agents, C07* Carvedilol, C07AG02 *Calcium channel blockers, C08* Gallopamil, C08DA02 Nicardipine, C08CA04 Felodipine, C08CA02 Nitrendipine, C08CA08 Bepridil, C08EA02 *Agents acting on the renin-angiotensin system, C09* Captopril, C09AA01 *Sex hormones and modulators of the genital system, G03* Mifepristone, G03XB01 *Psychoanaleptics, N06* Desipramine, N06AA01 *Antiprotozoals, P01* Mepacrine/Quinacrine, P01AX05 Quinine, P01BC01 *Ectoparasiticides, incl. scabicides, insecticides and repellents, P03* Disulfiram, P03AA04	*Cardiac therapy agents, C01* Quinidine, C01BA01 *Diuretics, C03* Conivaptan, C03XA02 *Immunosuppressants, L04* Sirolimus, L04AA10 *Analgesics, N02* Pentazocine, N02AD01 *Psycholeptics, N05* Chlorpromazine, N05AA01 *Antivirals for systemic use, J05* Saquinavir, J05AE01 Ritonavir, J05AE03 Indinavir, J05AE02 Amprenavir, J05AE05 *Antineoplastic agents, L01* Vinblastine, L01CA01 *Endocrine therapy, L02* Tamoxifen, L02BA01 *Cardiac therapy agents, C01* Nicardipine, C08CA04 *Antineoplastic agents, L01* Chlorambucil, L01AA02 Methotrexate, L01BA01 Cisplatin, L01XA01 Hydroxyurea/ hydroxycarbamide, L01XX05 *Endocrine therapy, L02* Tamoxifen, L02BA01 *Antigout agents, M04* Probenecid, M04AB01
P-gp substrate/inducer	**P-gp inducer/inhibitor**	
Antineoplastic agents, L01 Vincristine, L01CA02 Doxorubicin, L01DB01 Daunorubicin, L01DB02 Mitoxantrone, L01DB07 Etoposide, L01CB01 *Antigout agents, M04* Colchicine, M04AC01	*Cardiac therapy agents, C01* Amiodarone, C01BD01 *Antihypertensives, C02* Reserpine, C02AA02	

Appendix 1.2: The risk of specific anomalies among cases of individual drug with association and the risk determination from the comparison with the reference population

Types of specific anomalies	Drugs with associations	Number of users, n (%)		OR (95% CI)	p value†
		Case of specific anomalies (N_s)	Reference population (N=25,126)		
Heart anomalies (N_s=1244)	Cimetidine	2 (0.2)	20 (0.08)	2.02 (0.47-8.66)	0.28
	Ranitidine	3 (0.2)	142 (0.6)	0.43 (0.14-1.34)	0.17
Genital (N_s=406)	Omeprazole	8 (2)	295 (1.2)	1.69 (0.83-3.44)	0.16
	Pantoprazole	1 (0.2)	36 (0.1)	1.72 (0.24-12.58)	0.45
Respiratory (N_s=84)	Morphine	1 (1.2)	3 (0.01)	100.9 (10.39-979.94)	0.013
Musculoskeletal (N_s=1,054)	Haloperidol	3 (0.3)	25 (0.1)	2.87 (0.86-9.51)	0.1
	Quetiapine	1 (0.1)	21 (0.1)	1.14 (0.15-8.45)	0.6
	Risperidone	1 (0.1)	14 (0.1)	1.70 (0.22-12.97)	0.46
Nervous system (N_s=347)	Fluoxetine	1 (0.3)	110 (0.4)	0.66 (0.092-4.72)	1.0
	Citalopram	4 (1.2)	101 (0.4)	2.89 (1.06-7.9)	0.056
	Paroxetine	4 (1.2)	294 (1.2)	0.99 (0.37-2.66)	1.0
	Sertraline	1 (0.3)	35 (0.1)	2.07 (0.28-15.17)	0.39
	Fluvoxamine	1 (0.3)	55 (0.2)	1.32 (0.18-9.55)	0.54

†Fisher's exact test

APPENDICES FOR CHAPTER 3

Appendix 2.1: The codings for each type of major anomalies included in the study

Types of major anomalies	ICD 9	ICD 10
Nervous system	740-742	Q00-Q07 (not Q0782)
Eye, ear, face & neck	743-744 (not 74365, 74411-74413, 7443, 74491)	Q10-Q16, Q178, Q183, Q188 (not Q101-Q103, Q105, Q135)
Heart	745-746,7470-7474 (not 74550)	Q20-Q26 (not Q2111)
Respiratory	748 (not 74819, 748304, 74832, 74862)	Q30-Q34 (not Q309, Q314, Q315, Q320, Q331)
Oro-facial clefts	7490-7492	Q35-Q37
Digestive system, excluding diaphragmatic hernia and including hypertrophic pyloric stenosis	750-751 (not 7500, 75012, 75024, 7506, 7510)	Q38-Q45 (not Q381, Q382, Q3850, Q401, Q4021, Q430, Q4320, Q4381, Q4382)
Urinary	75261, 753 (not 753101, 75334), 756721	Q60-Q64 (not Q610, Q633), Q794
Genital	750-7524 (not 752431, 752442), 75260, 7527-7529	Q50-Q52, Q54-Q56 (not Q544, Q5520, Q5521)
Limb	75430, 75444, 75450, 75451, 754731, 7548, 7550-7554, 755505-755507, 75551, 755522, 75553, 755552-75559, 755612-7559	Q65-Q74 (not Q653-Q656, Q662-Q669, Q67, Q680, Q6821, Q683-Q685, Q7400)

Appendix 2.2: List of placental transporter proteins included in the study and the respective drug substrates/inducers/inhibitors

Placental transporters (genes)	Localization	Efflux or influx	Expression level*	Substrates	Substrates/Inhibitors	Inhibitors	Inducers
Breast cancer resistance protein, BCRP *(ABCG2)*	Apical	Efflux	0.0954	Cimetidine, Abacavir, Lamivudine, Prazosin, Rosuvastatin, Glibenclamide, Cervivastatin, Zidovudine, Methotrexate, Furosemide, Etoposide, Daunorubicin, Doxorubicin, Epirubicin, Grepafloxacin, Erlotinib, Lapatinib, Topotecan, Irinotecan, Flavonoids, Nitrofurantoin, Ciprofloxacin, Ofloxacin, Norfloxacin, Ulifoxacin, Erythromycin, Albendazole, Pitavastatin, Hydrochlorothiazide, Triamterene, Dantrolene, Riboflavin, Adefovir, Sulfasalazine	Lopinavir, Delavirdine, Efavirenz, Atazanavir, Imatinib, Gefitinib	Dipyridamole, Saquinavir, Ritonavir, Nelfinavir, Cyclosporine, Tamoxifen, Tacrolimus, Sirolimus, Nicardipine, Nitrendipine, Nimodipine, Reserpine, Omeprazole	
Organic cation transporter 3, OCT3 *(SLC22A3)*	Basolateral	Influx	0.146	Epinephrine, Histamine, Norepinephrine, Amiloride, Amphetamines	Cimetidine, Impiramine, Despiramine, Clonidine, Procainamide, Flecainide, Amiodarone, Verapamil, Diltiazem, Citalopram, Amitriptyline, Cisplatine, Oxaliplatine, Abacavir, Emtricitabine, Saquinavir, Tenofovir, Lamivudine, Ritonavir, Indinavir, Nelfinavir, Ranitidine, Metformin, Cocaine, Fexofenadine,	Quinidine, Rifampicin, Prazosin, Phenoxybenzamine, Progesterone, Amantadine, Ketamine, Memantine, Quinine, Atropine, Diphenhydramine, Etilefrine, Famotidine, Pentamidine, Phenformin	
Multidrug resistance-associated protein 1, MRP1 *(ABCC1)*	Basolateral	Efflux	0.0224	Cisplatin, Methotrexate, Etoposide, Vincristine, Vinblastine, Daunorubicin, Doxorubicin, Epirubicin, Folic Acid, Grepafloxacin, Glutathione, Leukotrien	Abacavir, Emtricitabine, Tenofovir, Lamivudine, Ritonavir, Indinavir, Lopinavir, Delavirdine, Efavirenz, Nevirapine, Atazanavir	Cyclosporine, Probenecid, Sulfinpyrazone, Indometachin	Saquinavir

Organic anion transporter 4, OAT4 *(SLC22A11)*	Basolateral	Influx	0.106		Pravastatin, Tetracycline, Zidovudine, Valproic acid, Methotrexate, Ketoprofen	Probenecid, Captopril, Olmesartan, Telmisartan, Candesartan, Losartan, Pratosartan, Valsartan, Ethacrynate, Furosemide, Torasemide, Bumetanide, Acetazolamide, Trichlormethiazide, Chlorothiazide, Cefadroxil, Cefazolin, Cefoperazone, Cefotaxime, Ceftriaxone, Diflunisal, Phenylbutazone, Sulfinpyrazone
Organic anion transporting polypeptide 2B1, OATP2B1 *(SLCO2B1)*	Basolateral	Influx	0.0557	Fluvastatin, Rosuvastatin, Benzylpenicillin, Bosentan, Unoprostone	Saquinavir, Nelfinavir, Fexofenadine, Atorvastatin, Pravastatin, Glibenclamide, Repaglinide, Lopinavir, Estrone-3-sulfate	Ritonavir, Indinavir, Rifampicin, Rifamycin, Cyclosporine, Gemfibrozil, Cerivastatin, Paclitaxel, Rosiglitazone, Simvastatin
Equilibrative nucleoside transporter, ENT1 *(SLC29A1)*	Apical	Influx	0.0656	Adenosine, Cladribine, Clofarabine, Gemcitabine, Ribavirin, Tiazofurin		Dipyridamole, Dilazep
Monocarboxylate transporter 1, MCT1 *(SLC16A1)*	Basolateral	Influx	0.091	Dipyridamole, Dilazep, Atorvastatin, Pravastatin, Valproic acid, Salicylic acid		Ketoprofen, Probenecid, Ibuprofen, Gabapentin
Monocarboxylate transporter 4, MCT4 *(SLC16A3)*	Apical	Influx	4.99	Valproic acid		Atorvastatin, Fluvastatin, Cerivastatin, Simvastatin, Lovastatin
Monocarboxylate transporter 8, MCT8 *(SLC16A2)*	Apical	Influx	0.0442			Probenecid, Ketoprofen, Despiramine
Monocarboxylate transporter 10, MCT10 *(SLC16A10)*	Apical	Influx	0.0203			Probenecid, Ketoprofen, Despiramine

*ratio of transporter protein mRNA to PPIA (peptidylprolyl isomerase A) mRNA

APPENDICES FOR CHAPTER 4

Appendix 3.1: Functional polymorphisms of transporter proteins and minor allele frequencies (MAFs) in different ethnicities

Gene (Protein)	SNPs	Exon/ Intron	dbSNP ID	Amino acid substitution	MAFs		
					Caucasians	African American	Asians
ABCB1 (P-gp)	T-129C	5ÚTR	rs3213619	-	0.059	NA	0.016-0.043
	A61G	2	rs9282564	Asn21Asp	0.08-0.112	0.025	NA
	T266C	5	rs35810889	Met89Thr	0.0005	0	NA
	G571A	7	NA	Gly191Arg	0.064	NA	NA
	G1199A/T	11	rs2229109	Ser400Asn/Ile	0.055-0.065	0	0
	C1236T	12	rs1128503	Gly412Gly	0.378-0.459	0.209	0.605-0.719
	GT1292-3TG	Walker A	NA	Cys431Leu	0.0135	NA	NA
	T1985G	17	rs35657960	Leu662Arg	0	0	0
	C2005T	17	rs35023033	Arg669Cys	0	0	0
	G2677T/A	21	rs2032582	Ala893Ser/Thr	0.355-0.416/0.01-0.019	NA	0.44-0.598/0.033-0.125
	T3322C	27	rs35730308	Trp1108Arg	0	0.002	NA
	T3421A	26	rs2229107	Ser1141Thr	0.05	0.111	NA
	C3435T	26	rs1045642	Ile1145Ile	0.48-0.57	0.10-0.202	0.383-0.66
	G3751A	29	rs28364274	Val1251Ile	0	0	0.002
ABCG2 (BCRP)	C34A	2	rs2231137	Val12Met	0.03-0.065	0.06	0.15-0.45
	C376T	4	rs72552713	Gln126Stop	0.0	0.0	0.01-0.02
	C421A	5	rs2231142	Gln141Lys	0.11-0.12	0.02-0.05	0.15-0.35

ABCC2 (MRP2)	C-24T	5ÚTR	rs717620	-	0.183	NA	0.181-0.229
	G1249A	10	rs2273697	Val417Ile	0.13-0.211	0.14	0.005-0.125
	C2366T	18	rs56220353	Ser789Phe	0.0	NA	0.007-0.01
	C3972T	28	rs3740066	Ile1324Ile	0.342-0.365	NA	0.0-0.222
	G4348A	31	rs56296335	Ala1450Thr	0	NA	0-0.01
	C4430T	31	rs142573385	Thr1477Met	0.002	0	NA
	G4544A	32	rs8187710	Cys1515Tyr	0.044-0.05	0.17	0-0.007
SLCO1B1 @ *SLC21A6* (SLCO1B1 @ OATP-C)	A388G	4	rs2291073	Asn130Asp	0.3-0.515	0.74	0.416-0.87
	T521C	5	rs4149056	Val174Ala	0.12-0.225	0.02	0.007-0.158
SLCO1B3 @ *SLC21A8* (SLCO1B3 @ OATP8)	T334G	3	rs4149117	Ser112Ala	0.523-0.88	0.386-0.41	0.62-0.92
	G699A	6	rs7311358	Met233Ile	0.71-0.87	0.41-0.478	0.64-0.9
	C767G	7	rs60140950	Gly256Ala	0-0.178	0.033	0-0.0.05
SLCO2B1 (OATP2B1)	G-282A	Promoter	rs73598371	-	NA	NA	0.544
	G935A	7	rs12422149	Arg312Gln	0.136	NA	NA
SLC22A1 (OCT1)	C41T		rs34447885	Ser14Phe	NA	0.031	0.0
	C181T	1	rs12208357	Arg61Cys	0.05-0.09	0.0	0.0
	C350T	1	rs200684404	Pro117Leu	0	0	0.023
	C480G	2	rs683369	Phe160Leu	0.005-0.251	0.02-0.032	0.00.13
	C566T	3	rs34104736	Ser189Leu	0.001	0.0	0.0
	C616T	3	NA	Arg206Cys	NA	NA	0.008
	G659T	3	rs36103319	Gly220Val	0.0-0.005	0.0-0.002	0.0
	C848T	5	rs4646277	Pro283Leu	0	NA	0-0.013
	C859G	5	rs4646278	Arg287Gly	0	0	0
	C1022T	6	rs2282143	Pro341Leu	0-0.02	0.08-0.082	0.089-0.168
	G1201A	7	rs34130495	Gly401Ser	0.011-0.39	0.007-0.27	0.0-0.22
	A1222G	7	rs628031	Met408Val	0.38-0.598	0.71-0.735	0.74-0.84

	A1258del or 1258-1260delATG	7	rs202220802	Met420del	0.18	0.3	0.0
	G1393A	9	rs45476695	Gly465Arg	0.0-0.04	0.0-0.016	0.0-0.04
SLC22A3 (OCT3)	G346T	1	rs8187717	Ala116Ser	0	0.025	0
	C1199T	7	rs8187725	Thr400Ile	0.004	NA	0.012
	C1316T	8	rs12212246	Ala439Val	0.004	NA	0.012
SLC22A4 (OCTN1)	C1507T	9	rs1050152	Leu503Phe	0.39-0.43	0.04	0.001-0.07
SLC22A5 (OCTN2)	G-207C	Promoter	rs2631367	-	0.463-0.5	0.382	0
	C51G	1	rs11568520	Phe17Leu	0.004	NA	NA
	G364T	1	rs201082652	Asp122Tyr	0.0	0.0	NA
	C430T	2	rs10040427	Leu144Phe	0	0.075	0
	A904G	5	rs75783492	Lys302Glu	0.0	0.0	0.003
	G1441T	8	rs11568513	Val481Phe	0.0	0.0	0.0
	C1645T	10	rs11568525	Pro549Ser	0	0.1	0
SLC47A1 (MATE1)	G191A	2	rs77630697	Gly64Asp	0-0.003	0	0.005-0.007
	C373T	4	rs77474263	Leu125Phe	0	0	0.003-0.007
	T404C	4	rs35646404	Thr159Met	0	0	0.01
	C929T	11	rs111060526	Ala310Val	0	0	0.022
	A983C	11	rs149774861	Asp328Ala	0	0	0.003-0.006
	G1012A	11	rs35790011	Ala338Ile	0	0.05-0.057	0-0.01
	A1421G	16	rs111060528	Asn474Ser	NA	NA	0.006
	G1438A	16	rs76645859	Val480Met	0	0	0.002-0.008
	G1557C	17	rs35395280	Cys497Ser	0	0.008-0.016	0
SLC47A2 (MATE2)	G192T	2	rs111060529	Lys64Asn	NA	NA	0.006
	632_633GC>TT	8	rs111060532	Gly211Val	NA	NA	0.017

NA, not available

Appendix 3.2: Effect of polymorphisms in the human placenta: mRNA and protein expression

Polymorphisms	Population	N	Sample tissue	Effect on mRNA level	Effect on protein expression	Ref.
ABCB1 **(P-gp)**						
T-129C	Japanese	89	Full-term placenta(enriched trophoblasts)	NA	TC<TT (2 fold, p= 0.002)	(1)
C1236T	Caucasians, African American, Hispanic	199	Brush border membrane vesicles from term placentas (38-41 weeks)	NA	TT=CT<CC (p<0.05)	(2)
G2677T/A	Japanese	89	Full-term placenta (enriched trophoblasts)	NA	T,A<G alleles (not significant)	(1)
	Caucasians, mothers		Term placenta (37-42 weeks)-whole placental tissue	N=35 NS	N=67 NS	(3)
	Caucasians, fetuses		Term placenta (37-42 weeks)-whole placental tissue	N=35 NS	N=73 NS	(3)
	Caucasians	44	Term placenta (38-42 weeks)- used intact cotyledons for perfusion process	NA	NS	(4)
	Caucasians, African American, Hispanic	199	Brush border membrane vesicles from term placentas (38-41 weeks)	NA	TT=CT<CC (p<0.05)	(2)
C3435T	Japanese	89	Full-term placenta(enriched trophoblasts)	NA	NS	(1)
	Japanese	96	Full-term placenta (highly enriched trophoblasts)	TT<CC, p=0.05 TT<CT, p=0.012	NA	(5)
	Caucasians, mothers		Term placenta (37-42 weeks)-whole placental tissue	*N=35* NS	*N=73* TT<CT<CC (p=0.049)	(3)
	Caucasians, fetuses		Term placenta (37-42 weeks)-whole placental tissue	*N=35* NS	*N=73* NS	(3)
	Caucasians, mothers and fetuses	39	Term placenta (37-42 weeks)-whole placental tissue	NA	TT/tt < CT/ct <CC/cc (p=0.01)	(3)
	Caucasians	44	Dually perfused term placenta (38-42 weeks)- used intact cotyledons for perfusion process	NA	TT>CC (p=0.009)	(4)
	Caucasians, African American, Hispanic	199	Brush border membrane vesicles from term placentas (38-41 weeks),	NA	TT=CT<CC (p<0.05)	(2)

G2677T/ C3435T	Caucasians, mothers	35	Term placenta (37-42 weeks)-whole placental tissue	*N=35* 2677T/3435T carriers showed a lower expression (p=0.02)	*N=67* 2677T/3435T carriers showed a lower expression (p=0.04)	(3)
	Caucasians, fetuses	69	Term placenta (37-42 weeks)-whole placental tissue	NA	NS	(3)
	Caucasians, fetuses	27	Term placenta (37-42 weeks), with homozygous haplotypes (GG2677/CC3435 vs TT2677/TT3435)	NA	NS GG/CC=TT/TT	(3)
	Caucasians, mothers and fetuses	9	Term placenta (37-42 weeks), with homozygous haplotypes (GG2677gg2677/CC3435cc3435 vs TT2677tt2677/TT3435tt3435	NA	NS GGgg/CCcc=TTtt/TTtt	(3)
ABCG2 **(BCRP)**						
C376T	Japanese	99	Term placenta (highly enriched placental trophoblasts)	NS	NS	(6)
C421A	Japanese	99	Term placenta (highly enriched placental trophoblasts)	NS	AA< CA<CC (p<0.05)	(6)
ABCC2 **(MRP2)**						
C-24T	Caucasian	58	Human placenta (chorionic villous tissues)	NS	NS	(7)
G1249A	Caucasian	58	Human placenta (chorionic villous tissues)	*Preterm:* (n=26) AA<GA<GG (p=0.044) *Term:* (n=32) NS	*Preterm:* (n=26) NS* *Term:* (n=32) NS	(7)
C3972T	Caucasian	58	Human placenta (chorionic villous tissues)			(7)

NA, not available; NS, not significant

***** a trend of lower expression in variant genotypes is observed

Appendix 3.3: Effect of polymorphisms in the human placenta: transplacental transfer/transport of drug substrates through placenta

Polymorphisms	Population	N	Sample tissue	Drug substrate	Measurement of transport	Effect of polymorphism	Ref.
ABCB1 **(P-gp)**							
C1236T	Caucasians, African American, Hispanic	105	Placental brush border membrane vesicle	Paclitaxel	Transport activity	TT>CC (p=0.04)	(2)
G2677T/A	Caucasians	20	Term placenta (38-42 weeks)-used intact cotyledons for perfusion process	Saquinavir	Transplacental transfer	NS[a]	(8)
	Caucasians	17	Term placenta (38-42 weeks)-used intact cotyledons for perfusion process	Quetiapine	Transplacental transfer	T>C (p=0.04)	(9)
	Caucasians, African American, Hispanic	105	Brush border membrane vesicles from term placentas (38-41 weeks)	Paclitaxel	Transport activity	NS (trend of A/A,A/T,T/T > CC, p=0.2)	(2)
C3435T	Caucasians	20	Term placenta (38-42 weeks)- used intact cotyledons for perfusion process	Saquinavir	Transplacental transfer	NS[a]	(8)
	Caucasians	17	Term placenta (38-42 weeks)- used intact cotyledons for perfusion process	Quetiapine	Transplacental transfer	T>C (p=0.04)[a]	(9)
	Caucasians	44	Dually perfused term placenta (38-42 weeks)- used intact cotyledons for perfusion process	Saquinavir	Transplacental transfer	NS[a]	(4)
	Caucasians, African American, Hispanic	105	Brush border membrane vesicles from term placentas (38-41 weeks)	Paclitaxel	Transport activity	TT>CC (p=0.02)	(2)
SLCO1B3 **(OATP1B3)**							
C767G	Finnish	12	Term placenta (38-40 weeks)	Repaglinide	Transplacental transfer	*Maternal-to-fetal:* CG (n=3)=GG(n=3), *Fetal-to-maternal:* CG(n=1)=GG(n=5)	(10)

T334G/ G699A	Finnish	12	Term placenta (38-40 weeks)	Repaglinide	Transplacental transfer	*Maternal-to-fetal:* GG/699AA(n=3)= 334TG/699GA(n=3), *Fetal-to-maternal:* 334TG/699GA(n=4)> 334GG/699AA(n=2), p=0.09	(10)
SLCO2B1 **(OATP2B1)**							
G-282A	Finnish	12	Term placenta (38-40 weeks)	Repaglinide	Transplacental transfer	*Maternal-to-fetal:* GA(n=1)=AA(n=5), *Fetal-to-maternal:* GG(n=1)=GA(n=1)=AA(n=4)	(10)
G935A	Finnish	12	Term placenta (38-40 weeks)	Repaglinide	Transplacental transfer	Maternal-to-fetal (n=6): GG(n=5)=AA(n=1)	(10)
SLCO1B1 **(OATP1B1)**				Repaglinide	Transplacental transfer		
A388G	Finnish		Term placenta (38-40 weeks)	Repaglinide	Transplacental transfer	*Maternal-to-fetal:* AA(n=2)=AG(n=2)=GG(n=2), *Fetal-to-maternal:* AA(n=2)=AG(n=3)=GG(n=1)	(10)
T521C	Finnish	12	Term placenta (38-40 weeks)	Repaglinide	Transplacental transfer		(10)

NS, not significant

[a]no correlation found between P-gp expression and transplacental transfer of drug substrae

REFERENCES

1. Tanabe M, Ieiri I, Nagata N, et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. J. Pharmacol. Exp. Ther. 297(3), 1137–1143 (2001).

2. Hemauer SJ, Nanovskaya TN, Abdel-Rahman SZ, et al. Modulation of human placental P-glycoprotein expression and activity by MDR1 gene polymorphisms. Biochem. Pharmacol. 79(6), 921–925 (2010).

3. Hitzl M, Schaeffeler E, Hocher B, et al. Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). Pharmacogenetics. 14(5), 309–318 (2004).

4. Rahi M, Heikkinen T, Hakkola J, et al. Influence of adenosine triphosphate and ABCB1 (MDR1) genotype on the P-glycoprotein-dependent transfer of saquinavir in the dually perfused human placenta. Hum. Exp. Toxicol. 27(1), 65–71 (2008).

5. Takane H, Kobayashi D, Hirota T, et al. Haplotype-Oriented Genetic Analysis and Functional Assessment of Promoter Variants in the MDR1 (ABCB1) Gene. J. Pharmacol. Exp. Ther. 311(3), 1179–1187 (2004).

6. Kobayashi D, Ieiri I, Hirota T, et al. Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. Drug Metab. Dispos. 33(1), 94–101 (2005).

7. zu Schwabedissen HEM, Jedlitschky G, Gratz M, et al. Variable expression of MRP2 (ABCC2) in human placenta: influence of gestational age and cellular differentiation. Drug Metab. Dispos. 33(7), 896–904 (2005).

8. Molsa M, Heikkinen T, Hakkola J, et al. Functional role of P-glycoprotein in the human blood-placental barrier. Clin. Pharmacol. Ther. 78(2), 123–131 (2005).

9. Rahi M, Heikkinen T, Härtter S, et al. Placental transfer of quetiapine in relation to P-glycoprotein activity. J. Psychopharmacol. 21(7), 751–756 (2007).

10. Tertti K, Petsalo A, Niemi M, et al. Transfer of repaglinide in the dually perfused human placenta and the role of organic anion transporting polypeptides (OATPs). Eur. J. Pharm. Sci. 44(3), 181–186 (2011).

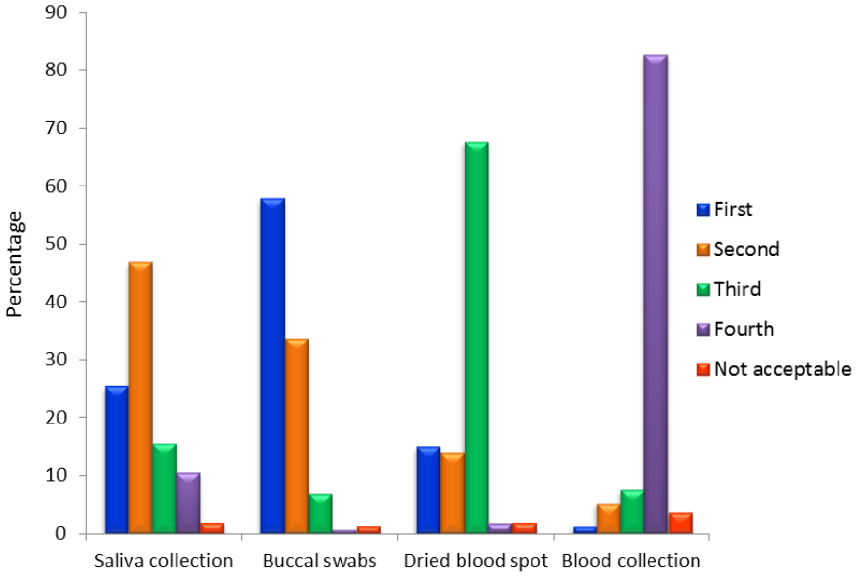
Appendix 3.4: Phenotypes of transporter polymorphisms based on *in vitro* transport of substrates in transfected cell lines

Phenotypes	Polymorphisms	Drugs/endogenous substrates	Transfected cell lines
ABCB1 (P-glycoprotein)			
Increase	G1199A	Vinblastine, vincristine, doxorubicin, paclitaxel , protease inhibitors	LLC-PK1, HEK
	T266C	Daunorubicin, doxorubicin	*Saccharomyces Cerevisiae* (yeast cells)
	T1985G		
	C2005T		
	T3421A		
Normal	A61G	Paclitaxel	HEK293
	G3751A		
	G2677A		
Decrease	G1199T	Doxorubicin, vinblastine, vincristine, paclitaxel	HEK
	G571A	Vinblastine, vincristine, paclitaxel, and etoposide	HEK
	GT1292-3TG (Cys431Leu)	Doxorubicin, paclitaxel, vinblastine, vincristine	HEK cells
	T3322C	Daunorubicin, doxorubicin	*Saccharomyces Cerevisiae* (yeast cells)
ABCC2 (MRP2)			
Increase	C4430T	TUDC	Sf9 membrane vesicles
Normal	G1249A	LTC4, E23G, E217G, TUDC, DNP-SG	Sf9 membrane vesicles , LLC-PK1 membrane vesicles
	C4430T	LTC4, E217G	Sf9 membrane vesicles
Decrease	C2366T	LTC4, E23G, E217G, TUDC	Sf9 membrane vesicles
	G4348A	LTC4, E23G, TUDC	Sf9 membrane vesicles
	C4430T	E23G	Sf9 membrane vesicles
	G4544A	Lopinavir	HEK293
SLC22A1 (OCT1)			
Normal	C480G	MPP+, TEA	*Xenopus laevis* oocytes
	C848T , C1022T	Metformin	Oocytes
	A1222G	MPP+, metformin	*Xenopus laevis* oocyte, HEK293
	A1258del	MPP+	*Xenopus laevis* oocyte
	A1222G/ A1258del*	Imatinib	KCL22
Decrease	C41T	Metformin	HEK293
	C181T	Metformin, Tropisetron	HEK293
	C848T, C1022T	Lamivudine	Oocytes
	C1022T	TEA	HEK293
	C289A, C350T, C616T	Metformin	HEK293
	C566T	Metformin	HEK293

	G659T	Metformin	HEK293
	G1201A	Metformin	HEK293
	A1258del	Metformin, tropisetron, imatinib	HEK293, KCL22
	G1393A	Metformin	HEK293
Diminish	C848T, C859G	TEA	HEK293
SLC22A3 (OCT3)			
Decrease	G346T, C1199T, C1316T	Histamine and MPP +	HEK293
SLC22A4 (OCTN1)			
Increase	C1507T	TEA, verapamil, cimetidine, lidocaine, quinidine	HEK293 fibroblast
Decrease	C1507T	Carnitine	HEK293 fibroblast
		Acetylcholine	*E.coli* proteoliposomes
SLC22A5 (OCTN2)			
Increase	G-207C	Carnitine	Ileal brush border membrane vesicles
Normal	C430T, C1645T	L-carnitine and TEA	HEK 293
Decrease	G-207C	Carnitine	Lymphoblastoid
	G1441T, C51G,	L-carnitine and TEA	HEK 293
	G364T, A904G	L-carnitine	HEK 293
SLC47A1 (MATE1)			
Increase	G1557C	Paraquat, oxaliplatin	HEK-293
Normal	C373T, G1012A	Oxaliplatin	HEK-293
Decrease	G191A, G1438A	Oxaliplatin	HEK-293
	T404C, G1012A	Metformin and TEA	HeLa
	G191A, C929T, A983C, G1012A, A1421G, G1557C	Metformin and TEA	HEK293
	C373T	Metformin, TEA, paraquat	HEK-293
Diminish	G191A	Metformin, TEA, paraquat	HEK293
	G1438A	Paraquat	HEK293
SLC47A2 (MATE2)			
Decrease	G192T	Metformin and TEA	HEK293
Diminish	632_633GC>TT	Metformin and TEA	HEK293

DNP-SG, 2,4-dinitrophenyl-S-glutathione; E217G, estradiol-17b- glucuronide; E23G, estradiol-3-glucuronide; HEK; human embryonic kidney cell line; KCL22; myeloid leukemia cell line; LLC-PK1, porcine renal epithelial cell line; LTC4, leukotriene C4; MPP+, methylpyridinium; TEA, tetraethylammonium; TUDC tauroursodeoxycholic acid

APPENDICES FOR CHAPTER 5



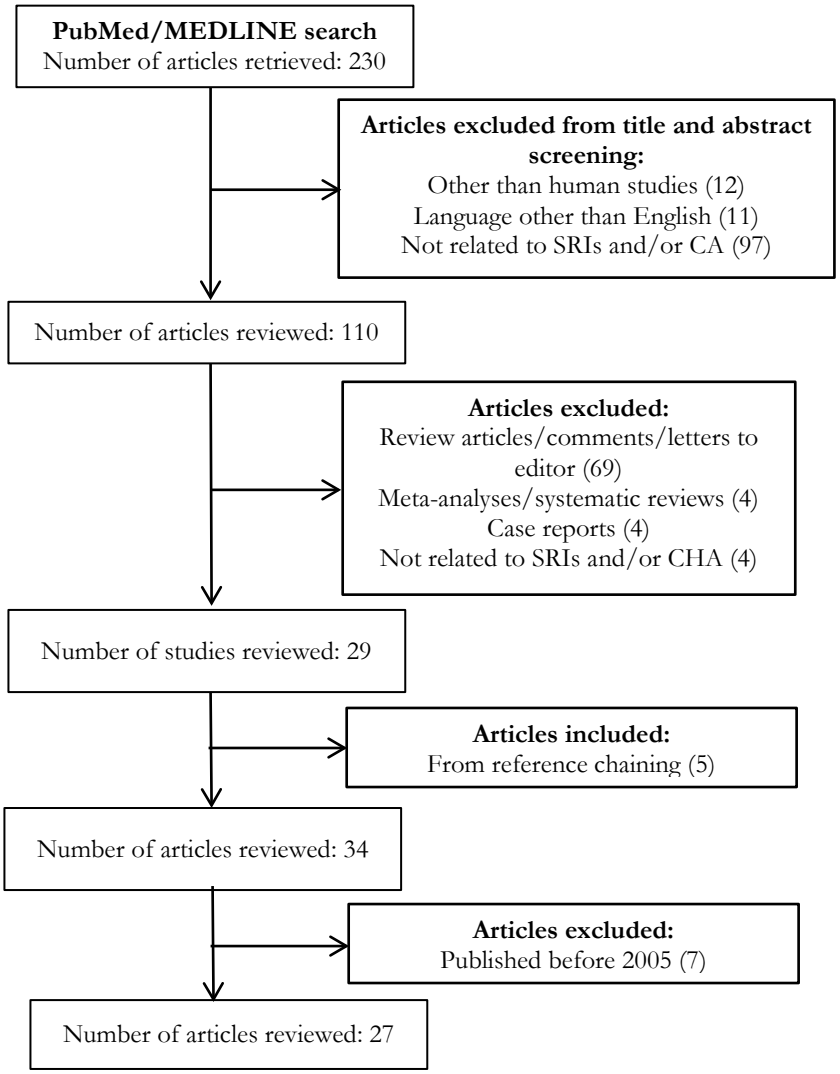
Appendix 4.1: Preferred DNA collection methods among respondents (n=173)

Appendix 4.2: The likelihood of having a good attitude towards pharmacogenetics testing given respondent characteristics

Characteristics	Gave positive answers to attitude questions, adjusted OR (95% CI) *, p value			
	Allows doctors to use DNA information in (future) drug treatment	Willing to get a DNA-test before a certain medication is prescribed	Allows pharmacogenetic information to be used in (future) drug treatment	Sum score 3 to attitude questions
Educational level				
Low	Reference level			
Middle	0.94 (0.28-3.15), 0.92	1.01 (0.33-3.12), 0.98	1.34 (0.47-3.80), 0.58	1.41 (0.51-3.88), 0.59
High	0.69 (0.21-2.29), 0.55	0.86 (0.28-2.63), 0.79	1.34 (0.47-3.80), 0.58	1.61 (0.58-4.43), 0.36
Living situation (Living with spouse/partner/ others vs. alone/ divorced)	0.30 (0.037-2.40), 0.2	0.58 (0.12-2.80), 0.49	0.87 (0.22-3.46), 0.89	0.47 (0.12-1.89), 0.29
Having chronic disease(s) † (Yes vs. No)	1.37 (0.66-2.85), 0.40	1.66 (0.80-3.44), 0.18	1.25 (0.64-2.45), 0.52	1.27 (0.68-2.38), 0.46
Having chronic disease(s)†† (Yes vs. No)	1.28 (0.69-2.38), 0.43	1.36 (0.74-2.48), 0.32	1.57 (0.88-2.82), 0.13	1.39 (0.81-2.39), 0.24
Used medication during pregnancy (Yes vs. No/Do not know)	0.71 (0.38-1.32), 0.28	1.27 (0.69-2.33), 0.45	1.17 (0.65-2.10), 0.60	0.86 (0.50-1.48), 0.58
Experienced side effect (Yes vs. No/Do not know)	0.86 (0.46-1.59), 0.63	1.57 (0.85-2.91), 0.15	1.51 (0.83-2.72), 0.18	1.24 (0.72-2.14), 0.44
Experienced side effect†† (Yes vs. No/Do not know)	0.90 (0.48-1.66), 0.73	1.25 (0.69-2.26), 0.47	1.40 (0.78-2.50), 0.26	1.0 (0.58-1.70), 0.97
Stopping medication due to side effect(s) (Yes vs. No/Do not know)	1.03 (0.53-1.98), 0.93	1.50 (0.78-2.87), 0.23	1.66 (0.88-3.13), 0.12	1.18 (0.67-2.10), 0.56
Stopping medication due to inefficacy (Yes vs. No/ Do not know)	1.11 (0.53-2.32), 0.78	0.69 (0.34-1.36), 0.28	0.89 (0.45-1.74), 0.73	0.85 (0.45-1.60), 0.62
Aware of the term 'pharmacogenetics'	1.52 (0.70-3.30), 0.29	1.2 (0.58-2.47), 0.62	1.66 (0.80-3.43), 0.18	1.17 (0.61-2.23), 0.64
Aware of the meaning of 'pharmacogenetics'	1.65 (0.68-4.01), 0.27	1.38 (0.61-3.12), 0.44	1.34 (0.61-2.97), 0.47	1.15 (0.56-2.34) 0.71
Knowledge about pharmacogenetics (sum score of 3 to knowledge questions vs. others)	**2.68 (1.43-5.04), 0.002**	**2.78 (1.50-5.14), 0.001**	**4.75 (2.56-8.81), <0.001**	**3.43 (1.90-6.17), <0.001**

*adjusted for age; †themselves; ††themselves or family members; bold font indicates significant associations.

APPENDICES FOR CHAPTER 6



Appendix 5.1: Flow-chart outlining the search strategy. A literature review was performed in May 2015 using the PubMed database. Articles are searched using the combinations of the following keywords: "Serotonin Uptake Inhibitors" [Mesh], "Congenital Abnormalities" [Mesh], and "humans" [MeSH Terms]. SRIs, serotonin reuptake inhibitors; CA, congenital anomalies; CHA, congenital heart anomalies

Appendix 5.2: Studies (2005-2015) that found an association between SRI use in early pregnancy and the risk of congenital anomalies including CHA

Authors	Study design (database used)	Location	Number of patients	SRIs/SSRIs	Definition of first trimester exposure	Outcome (anomalies) definition	Drawn conclusion
Be´rard et. al., 2015 (1)	Cohort study (pregnancy cohort)	Canada	18 493 pregnancies (2 329 exposed to SRIs)	Sertraline & other SSRIs	14 weeks of the first trimester (confirmed with ultrasound)	Major congenital anomalies detected within the first year of life	Infants exposed to sertraline were at increased risk of ASD and VSD
Wemakor et. al., 2015 (2)	Case-malformed control study (congenital anomaly registries)	12 European countries	42 983 cases and malformed controls (including TOP, miscarriages, stillbirths), 12 876 CHA cases	Any SSRIs without any other type of antidepressants	First day of the LMP up to 12th week of gestation	Major CHA (excluding preterm deliveries with only PDA and all cases with open foramen ovale)	Result supports teratogenic effects of SSRIs specific to certain anomalies, but cannot exclude confounding by indication or associated factors
Ban et. al., 2014 (3)	Cohort study (primary care records)	UK	325 294 women without depression, 7 683 women exposed to SSRIs	SSRIs alone, SSRIs + TCAs	1 month before to 3 months after conception	Major congenital anomalies, overall and specific	Paroxetine increases the risk of CHA. The risk of overall major congenital anomalies did not increase with maternal depression & antidepressants
Knudsen et. al., 2014 (4)	Cohort study (birth defects registry)	Denmark	845 exposed, 71 435 not exposed	SSRIs	30 days before LMP until 91 days after LMP	CHA detected within first 5 years of life (excluding chromosomal anomalies, genetic syndrome or microdeletion)	SSRI use increases the risk of severe CHD and socioeconomic status did not confound the risk
Polen et. al., 2013 (5)	Case-control study (NBDPS)	US	27 045 women. Exposed: 14/8002 (0.17%) of controls, 77/19 043 (0.4%) of cases	Venlafaxine	1 month before conception & first trimester (maternal interview after delivery)	30 selected birth defects	Associations found for certain birth defects, especially anencephaly, cleft palate, gastroschisis, and some CHD (ASD, coarctation of aorta, LVOTO)
Malm et. al., 2011 (6)	Retrospective cohort (national birth registers)	Finland	6 881 exposed, 618 727 not exposed	SSRIs	1 month before pregnancy & first trimester (based on LMP and U/S data)	Major congenital anomalies (does not exclude chromosomal abnormalities)	Associations found for specific CV anomalies; fluoxetine & isolated VSD, paroxetine & right ventricular outflow defect
Reis and Kallèn, 2010 (7)	Case-control study (national birth registers)	Sweden	14 821 exposed, 1 062 190 not exposed	Antidepressants	Early use: ~10-12 weeks, Later use: subsequent prescription	'Relatively severe malformations' excluding chromosomal abnormalities	Associations found between paroxetine and CV defects, and between SSRI and hypospadias (particularly with paroxetine)
Bakker et. al., 2010 (8)	Case-malformed control study (birth defect registry)	Netherlands	678 cases of isolated heart defects, 615 controls of chromosomal anomalies	Paroxetine	4 weeks before conception until 12th week of pregnancy	Major isolated CHA	Increased risk of isolated ASD with paroxetine use in the first trimester, but the absolute risk is small

Merlob et. al., 2009 (9)	Prospective study (birth defect surveillance database)	Israel	67 871 infants (235 exposed, 2 537 not exposed)	SSRIs	First trimester (based on maternal interview upon admission to maternity ward)	Cardiac murmur on the first day of life and persist on 2nd and 3rd day	Increased risk of mild, non-syndromic heart defects in infants exposed to SSRIs (small risk)
Pedersen et. al., 2009 (10)	Cohort study (national health & birth defect registries)	Denmark	1 370 exposed, 493 113 not exposed	SSRIs (two or more filled Rx)	28 days before to 112 days after gestation.	CHA detected within the first year of life (exclude stillbirth and multiple births)	Citalopram and sertraline were associated with an increased prevalence of septal heart defects (limited risk)
Diav-Citrin et. al., 2008 (11)	Prospective study (multi-centre TIS)	Israel, Italy, Germany	463 exposed to paroxetine, 346 exposed to fluoxetine, 1 467 control: exposed to non-teratogens	Paroxetine and fluoxetine	Paroxetine: between weeks 3-13, fluoxetine: between weeks 2-13 after LMP	Major congenital anomalies, including VSD, detected within the first 6 years of life	Possible association between CV anomalies and first trimester exposure to fluoxetine
Oberlander et. al., 2008 (12)	Cohort study (national health registries)	Canada	2 625 exposed, 107 320 not exposed	SRIs	Days of dosing covered by the Rx overlapped with the period from LMP to LMP + 90 days	Major congenital anomalies	The risk for cardiac anomalies increased when SRIs were used in combination with benzodiazepine (possible effect of drug interaction)
Cole et. al., 2007 (13)	Cohort study (insurance claim database)	US	paroxetine: 791 (monotherapy), 989 (mono or polytherapy); other antidepressants : mono (4 072), mono & poly (4 767)	Paroxetine mono- or poly therapy	Prescription duration overlapping with the earliest conception date until 91 days	All major congenital malformations detected within the first 9 months of age	Increased risk of overall congenital anomalies with paroxetine exposure during the first trimester compared to the use of other antidepressants
Louik et. al., 2007 (14)	Case-control study (birth defects surveillance database)	US	9 849 infants with and 5 860 infants without birth defects	SSRIs	28 days before to 112 days after the LMP	Overall and specific anomalies, major only	Increased risk for some specific anomalies with individual SSRIs use (small absolute risks)
Källén et. al., 2007 (15)	Cohort study (national birth registries)	Sweden	6 481 exposed, 873 876 not exposed	SSRIs	Detected during the first antenatal visit (90% before the end of week 12)	Overall anomalies (including minor conditions)	Paroxetine increased the risk of any cardiac anomalies, mostly of ASD & VSD
Berard et. al., 2007 (16)	Case-control study (national health registries)	Canada	1 403 women. 542 exposed to paroxetine,443 to other SSRIs, 418 to other antidepressants	Paroxetine	The first trimester of pregnancy (0-14 weeks of gestational age)	Any major anomaly including cardiac anomalies	Association found only in doses above 25 mg/day
Wogelius et. al., 2006 (17)	Cohort study (national birth registry)	Denmark	1 051 exposed, 150 780 not exposed	SSRIs	30 days before conception until the end of the first trimester	Congenital anomalies detected within the first year of life	A moderately increased risk of overall congenital anomalies was found with the use of SSRIs

Abbreviations: SRI, serotonin reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; CHA, congenital heart anomalies; ASD, atrial septal defect; VSD, ventricular septal defect; TOP, termination of pregnancy; LMP, last menstrual period; PDA, patent ductus arteriosus; NBDPS, the National Birth Defects Prevention Study; LVOTO, left ventricular outflow tract obstruction; U/S, ultrasound; TIS, Teratology Information Service.

Appendix 5.3: Studies (2005-2015) reporting no association between SRI use in early pregnancy and the risk of congenital anomalies including CHA

Authors	Study design	Location	Number of patients	SSRIs	Definition of first trimester exposure	Outcome (anomalies) definition	Drawn conclusion
Furu et. al., 2015 (18)	Cohort study and sibling design (national health registries)	Denmark, Finland, Iceland, Norway, and Sweden	2.3 million live singletons, 2 288 sibling cohort	All SSRIs, including venlafaxine	30 days before the first day of LMP until the end of the first trimester	Major cardiac and other anomalies diagnosed within 1 year after birth	Results did not suggest teratogenic effect of SSRIs and venlafaxine
Huybrechts et. al., 2014 (19)	Cohort study (national health registry)	US	46 144 exposed to SSRIs, 885 115 not exposed	SSRIs and other antidepressants	Days/duration of Rx supplied overlap with 90 days the first trimester	Any cardiac malformations	Do not support earlier findings of an association between antidepressants & cardiac anomalies
Vasilakis-Scaramozza et. al., 2013 (20)	Matched cohort study (general practice records)	UK	3 276 exposed, 6 617 non-exposed (singleton pregnancies)	TCA and SSRIs	At least one Rx of TCA or SSRIs from 180 to 335 days prior to delivery date for livebirth cases, and 70-225 days for stillbirths/TOP	Major congenital anomalies, detected before 1st birthday	Exposure to TCA or SSRIs does not increase the risk of congenital anomalies
Margulis et. al. 2013 (21)	Cohort study (national livebirth cohort)	UK	3 046 exposed, 8 991 not exposed	SSRIs	First trimester (assumption of pregnancy duration 273 days, for term, 258 days for preterm births)	Cardiac malformation detected in the first year 6 years of life	No association found between maternal use of SSRIs and cardiac anomalies
Klieger-Grossmann et. al., 2012 (22)	Observational cohort study (pregnancy surveillance registries)	Canada	6 582 mothers (212 mothers in each group of escitalopram users, other antidepressants, and non-teratogenic drug users)	Escitalopram	The first trimester of pregnancy	Major congenital malformations	Escitalopram was not associated with increased risk for major malformation
Einarson et. al., 2009 (23)	Matched-cohort study (teratogen information service)	Canada	928 each in the exposed and unexposed groups	Antidepressants (SSRIs, SNRIs)	First trimester (based on maternal interview)	Major congenital malformations (maternal interview, corroborated by physician report)	No increase in the risk of major malformations with the use of antidepressants as a group or individually

Wichman et. al., 2009 (24])	Retrospective cohort study (health registry)	US	808 exposed, 24 406 not exposed	SSRIs, venlafaxine	0-13 weeks of gestation	CHA (diagnosed at birth or before discharge), obstetric data and medical record	No associations between SSRI use and CHA
Lennestal et. al., 2007 (25)	Cohort study (national birth registries)	Sweden	860 215 deliveries (732 women used SNRI/NRI in early pregnancy)	SNRIs/NRIs (mianserin, mirtazapine, venlafaxine, reboxetine)	First trimester (interview during the first antenatal care visit)	Delivery outcome, including congenital anomalies	No increase in the risk of congenital anomalies in infants exposed to SNRI/ NRIs
Davis et. al., 2007 (26)	Retrospective cohort study (health registry)	US	1 047 exposed to SSRI at any time during pregnancy, 75 833 not exposed to any antidepressants	SSRI, TCAs	First 90 days of pregnancy (assumption of gestational age of 270 days before delivery date)	Congenital malformations & perinatal complications	No increase in the risk of cardiovascular anomalies with paroxetine use
Alwan et. al., 2007 (27)	Case-control study (NBDPS)	US	9 622 cases of congenital anomalies, 4 092 controls. 408 exposed to SSRIs from both groups	Any SSRIs	1 month before to 3 months after conception (date of conception: 266 days before the estimated date of delivery)	Major CHA, isolated and multiple anomalies	Maternal use of SSRIs was not associated with an increased risk of CHA or other birth defects

SRI, serotonin reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; CHA, congenital heart anomalies; LMP, last menstrual period; VSD, ventricular septal defect; TCA, tricyclic antidepressants; TOP, termination of pregnancy; SNRI, serotonin/noradrenaline reuptake inhibitor; NRI, noradrenaline reuptake inhibitor.

REFERENCES

1. Bérard A, Zhao J-P, Sheehy O. Sertraline use during pregnancy and the risk of major malformations. Am J Obstet Gynecol 2015; 212: e1–12.

2. Wemakor A, Casson K, Garne E, Bakker M, Addor M-C, Arriola L et al. Selective serotonin reuptake inhibitor antidepressant use in first trimester pregnancy and risk of specific congenital anomalies: a European register-based study. Eur J Epidemiol 2015; : (DOI)10.1007/s10654–015–0065–y.

3. Ban L, Gibson J, West J, Fiaschi L, Sokal R, Smeeth L et al. Maternal depression, antidepressant prescriptions, and congenital anomaly risk in offspring: A population-based cohort study. BJOG An Int J Obstet Gynaecol 2014; 121: 1471–1481.

4. Knudsen T, Hansen A, Garne E, Andersen A-M. Increased risk of severe congenital heart defects in offspring exposed to selective serotonin-reuptake inhibitors in early pregnancy – an epidemiological study using validated EUROCAT data. BMC Pregnancy Childbirth 2014; 14: 333.

5. Polen KN, Rasmussen SA, Riehle-Colarusso T, Reefhuis J, Study NBDP. Association between reported venlafaxine use in early pregnancy and birth defects, national birth defects prevention study, 1997-2007. Birth defects Res A, Clin Mol Teratol 2013; 97: 28–35.

6. Malm H, Artama M, Gissler M, Ritvanen A. Selective serotonin reuptake inhibitors and risk for major congenital anomalies. Obstet Gynecol 2011; 118: 111–120.

7. Reis M, Kallen B. Delivery outcome after maternal use of antidepressant drugs in pregnancy: an update using Swedish data. Psychol Med 2010; 40: 1723–1733.

8. Bakker MK, Kerstjens-Frederikse WS, Buys CH, de Walle HE, de Jong-van den Berg LT. First-trimester use of paroxetine and congenital heart defects: a population-based case-control study. Birth defects Res A, Clin Mol Teratol 2010; 88: 94–100.

9. Merlob P, Birk E, Sirota L, Linder N, Berant M, Stahl B et al. Are selective serotonin reuptake inhibitors cardiac teratogens? Echocardiographic screening of newborns with persistent heart murmur. Birth Defects Res A Clin Mol Teratol 2009; 85: 837–41.

10. Pedersen LH, Henriksen TB, Vestergaard M, Olsen J, Bech BH. Selective serotonin reuptake inhibitors in pregnancy and congenital malformations: population based cohort study. BMJ 2009; 339: b3569.

11. Diav-Citrin O, Shechtman S, Weinbaum D, Wajnberg R, Avgil M, Gianantonio ED et al. Paroxetine and fluoxetine in pregnancy: a prospective, multicentre, controlled, observational study. Br J Clin Pharmacol 2008; 66: 695–705.

12. Oberlander TF, Warburton W, Misri S, Riggs W, Aghajanian J, Hertzman C. Major congenital malformations following prenatal exposure to serotonin reuptake inhibitors and benzodiazepines using population-based health data. Birth defects Res B, Dev Reprod Toxicol 2008; 83: 68–76.

13. Cole JA, Ephross SA, Cosmatos IS, Walker AM. Paroxetine in the first trimester and the prevalence of congenital anomalies. Pharmacoepidemiol Drug Saf 2007; 16: 1075–1085.

14. Louik C, Lin AE, Werler MM, Hernandez-Diaz S, Mitchell AA. First-trimester use of selective serotonin-reuptake inhibitors and the risk of birth defects. N Engl J Med 2007; 356: 2675–2683.

15. Källén BAJ, Olausson PO. Maternal use of selective serotonin re-uptake inhibitors in early pregnancy and infant congenital malformations. Birth Defects Res Part A - Clin Mol Teratol 2007; 79: 301–308.

16. Berard A, Ramos E, Rey E, Blais L, St-Andre M, Oraichi D. First trimester exposure to paroxetine and risk of cardiac malformations in infants: the importance of dosage. Birth defects Res B, Dev Reprod Toxicol 2007; 80: 18–27.

17. Wogelius P, Nørgaard M, Gislum M, Pedersen L, Munk E, Mortensen PB et al. Maternal use of selective serotonin reuptake inhibitors and risk of congenital malformations. Epidemiology 2006; 17: 701–704.

18. Furu K, Kieler H, Haglund B, Engeland A, Selmer R, Stephansson O et al. Selective serotonin reuptake inhibitors and venlafaxine in early pregnancy and risk of birth defects: population based cohort study and sibling design. BMJ Open 2015; 350: h1798–h1798.

19. Huybrechts KF, Palmsten K, Avorn J, Cohen LS, Holmes LB, Franklin JM et al. Antidepressant use in pregnancy and the risk of cardiac defects. N Engl J Med 2014; 370: 2397–2407.

20. Vasilakis-Scaramozza C, Aschengrau A, Cabral H, Jick SS. Antidepressant use during early pregnancy and the risk of congenital anomalies. Pharmacotherapy 2013; 33: 693–700.

21. Margulis A V, Abou-Ali A, Strazzeri MM, Ding Y, Kuyateh F, Frimpong EY et al. Use of selective serotonin reuptake inhibitors in pregnancy and cardiac malformations: a propensity-score matched cohort in CPRD. Pharmacoepidemiol Drug Saf 2013; 22: 942–951.

22. Klieger-Grossmann C, Weitzner B, Panchaud A, Pistelli A, Einarson T, Koren G et al. Pregnancy Outcomes Following Use of Escitalopram: A Prospective Comparative Cohort Study. J Clin Pharmacol 2012; 52: 766–770.

23. Einarson A, Choi J, Einarson TR, Koren G. Incidence of major malformations in infants following antidepressant exposure in pregnancy: Results of a large prospective cohort study. Can J Psychiatry 2009; 54: 242–246.

24. Wichman CL, Moore KM, Lang TR, St Sauver JL, Heise RHJ, Watson WJ. Congenital heart disease associated with selective serotonin reuptake inhibitor use during pregnancy. Mayo Clin Proc 2009; 84: 23–27.

25. Lennestål R, Källén B. Delivery outcome in relation to maternal use of some recently introduced antidepressants. J Clin Psychopharmacol 2007; 27: 607–613.

26. Davis RL, Rubanowice D, McPhillips H, Rabael MA, Andrade SE, Smith D et al. Risk of congenital malformations and perinatal events among infants exposed to antidepressant medications during pregnancy. Pharmacoepidemiol Drug Saf 2007; 16: 1086–1094.

27. Alwan S, Reefhuis J, Rasmussen SA, Olney RS, Friedman JM, Study NBDP. Use of selective serotonin-reuptake inhibitors in pregnancy and the risk of birth defects. N Engl J Med 2007; 356: 2684–2692.

APPENDICES FOR CHAPTER 7

Appendix 6.1: List of study SNPs and the proposed/predicted functional effects

Number	Gene/ allele number	rs number	Chromosome location	Nucleotide change	Variant type	Variant allele frequency (European)[a]	Functional effects on expression/ activity
	CYP1A2						
1	*1W	rs2069521	15:74746626	-3113G>A	Upstream gene variant	0.02	Increased
2		rs2069526	15:74749000	-739T>G	Intronic	0.02	Increased
3		rs4646425	15:74750940	832-249 C>T	Intronic	0.02	Increased
4		rs4646427	15:74753351	1253+81 T>C	Intronic	0.02	Increased
5		rs2472304	15:74751897	1042+43 G>A	Intronic	0.6	Decreased
6		rs2470890	15:74755085	1548C>T	Synonymous	0.6	Decreased
	CYP2C9						
7	*2	rs1799853	10:94942290	430C>T	Missense	0.12	Decreased
8	*3	rs1057910	10:94981296	1075A>C	Missense	0.07	Decreased
9	*6	rs9332131	10:94949282	818delA	Frameshift	0.0	No activity
10	*15	rs72558190	10:94947782	485C>A	Stop codon	0.001	No activity
11	*4	rs56165452	10:94981297	1076T>A	Missense	0.002	Decreased
12	*5	rs28371686	10:94981301	1080C>G	Missense	0	Decreased
13	*8	rs7900194	10:94942309	449G>A	Missense	0	Decreased
14	*11	rs28371685	10:94981224	1003C>T	Missense	0	Decreased
15	*13	rs72558187	10:94941958	269T>C	Missense	0	Decreased
	CYP2C19						
16	*2	rs4244285	10:94781859	681G>A	Synonymous	0.15	No activity
17	*3	rs4986893	10:94780653	636G>A	Stop codon	0	No activity
18	*4	rs28399504	10:94762706	1A>G	Missense	0	No activity
19	*5	rs56337013	10:94852738	1297C>T	Missense	0.001	No activity
20	*6	rs72552267	10:94775453	395G>A	Missense	0	No activity
21	*7	rs72558186	10:94781999	19+2T>A	Splice donor variant	0.001	No activity
22	*8	rs41291556	10:94775416	358T>C	Missense	0	No activity
23	*9	rs17884712	10:94775489	431G>C	Missense	0	Decreased
24	*10	rs6413438	10:94781858	680C>T	Missense	0	Decreased
25	*17	rs12248560	10:94761900	-806C>T	Intronic	0.22	Increased

Appendix 6.1 (cont.)

Number	Gene/ allele number	rs number	Chromosome location	Nucleotide change	Variant type	Variant allele frequency (European)[a]	Functional effects on expression/ activity
	CYP2D6						
26	*2	rs16947	22:42127941	886G>A	Intronic/ Missense	0.34	Normal
27	*9	rs5030656	22:42128174	2615delAAG	Insertion/ deletion	0.03	Decreased
28	*10	rs1065852	22:42130692	100C>T	Missense	0.2	Decreased
29	*17	rs28371706	22:42129770	1023C>T	Missense	0.2	Decreased
30	*41	rs28371725	22:42127803	2988G>A	Intronic/ Missense	0.09	Decreased
31	*3A	rs35742686	22:42128242	622delA	Frameshift	0.02	No activity
32	*4	rs3892097	22:42128945	1846G>A	Splice acceptor variant	0.19	No activity
33	*6	rs5030655	22:42129084	1707delT	Intronic/ Frameshift	0.02	No activity
34	*7	rs5030867	22:42127856	818A>C	Intronic/ Missense	0	No activity
35	*8	rs5030865	22:42129033	505C>T/A	Intronic/ Missense	0	No activity
36	*11	rs5030863	22:42525912	883G>C	Splicing defect	NA	No activity
37	*12	rs5030862	22:42130668	124G>A	Intronic/ Missense	0	No activity
	ABCB1						
38		rs1128503	7:87550285	1236C>T	Synonymous	0.42	Decreased
39		rs2032582	7:87531302	2677G>T/A	Missense	0.41(T), 0.02(A)	Decreased
40		rs1045642	7:87509329	3435C>T	Synonymous	0.52	Decreased
41		rs2235040	7:87536434	2481+24G>A	Intronic	0.13	Decreased (?)
42		rs4148739	7:87531733	2482-236A>G	Intronic	0.13	Decreased (?)
43		rs1882478	7:87507702	3489+1573G>A	Intronic	0.26	Decreased (?)
44		rs9282564	7:87600124	61A>G	Missense	0.08	Decreased (?)
45		rs10256836	7:87571457	287-1234G>C	Intronic	0.3	Increased (?)
	SLC6A4						
46	SERTLPR	rs4795541	17:30237341	S and L-alleles	Insertion/ deletion	0.4	L: increased efficacy
47	SERT VNTR	rs57098334	17:30221570	9,10 or 12 tandem repeats	Intronic	0.47	12 repeat: increased efficacy

Appendix 6.1 (cont.)

Number	Gene/ allele number	rs number	Chromosome location	Nucleotide change	Variant type	Variant allele frequency (European)[a]	Functional effects on expression/ activity
	HTR1A						
48		rs1364043	5:63955024	63250851T>G (A/C)	Downstream gene variant	0.21	Increased response
49		rs6295	5:63962738	-1019G>C	Intronic	0.54	Increased response
	HTR1B						
50		rs6296	6:77462543	861G>C	Synonymous	0.26	Increased response
51		rs6298	6:77463275	129C>T	Synonymous	0.74	Reduced response
	HTR2A						
52		rs7997012	13:46837850	614-2211G>A	Intronic	0.43	Increased response/side effects
53		rs6313	13:46895805	102C>T	Synonymous	0.44	Increased response/side effects
54		rs6314	13:46834899	1354C>T	Missense	0.08	Increased response/side effects
55		rs1928040	13:46873101	613+19289C>T	Intronic	0.49	Increased response
56		rs6311	13:46897343	-1438G>A	Upstream gene variant	0.44	Reduced response/ side effects (A)
	HTR3B						
57		rs1176744	11:113932306	386A>C	Missense	0.31	Reduced side effects
58		rs3831455	11:113904828	-106_-104delGGA	5'UTR variant	NA	Increased side effects

a 1000Genome; NA, not available; UTR, untranslated region; S, small; L, long.

Appendix 6.2: Cases exposed to SRIs and their maternal CYP450 enzyme phenotypes

ID	Birth type	Types of CHA (ICD10 Code)	Use/intake during the first trimester			Types of SRIs used in the first trimester	Maternal CYP450 enzyme phenotypes			
			Tobacco	Alcohol	Folic acid		CYP1A2	CYP2C9	CYP2C19	CYP2D6
E01	Live birth	Q2112 (sinus venous defect)	Yes	Yes	Yes	paroxetine	decreased function	intermediate	rapid	**normal**
E02	Live birth	Q2120 (ASD type 1)	No	No	Yes	venlafaxine	decreased function	**normal**	**intermediate**	**normal**
E03	Live birth	Q2310 (bicuspid aortic valve + aortic valve stenosis)	No	Yes	Yes	paroxetine	normal	normal	normal	**normal**
E04	Live birth	Q251, Q2104 (CoA, perimem-branous VSD)	Yes	No	Yes	paroxetine venlafaxine	decreased function	**normal**	**normal**	**normal**
E05	Live birth then deceased	Q251, Q201, Q254 (CoA, Taussig Bing)	No	No	Yes	paroxetine	decreased function	normal	intermediate	**normal**
E06	Terminated pregnancy	Q234 (HLHS)	No	Yes	Yes	fluoxetine	decreased function	**normal**	normal	**normal**
E07	Live birth	Q2103 (muscular VSD)	No	No	Yes	fluoxetine	decreased function	**normal**	normal	**normal**

CHA, congenital heart anomalies; CYP, cytochrome P450; ASD, atrial septal defect; VSD, ventricular septal defect; CoA, coarctation of the aorta; HLHS, hypoplastic left heart syndrome; words in bold represent the relevant phenotypes associated with the metabolism of each SRI

SUMMARY

Drug use during pregnancy has been assessed in many drug utilization studies. These studies have shown that there is a high prevalence of pregnant women being prescribed at least one drug. The thalidomide tragedy in the 1960's has markedly improved the vigilance of drug use during pregnancy. Several pregnancy classification systems are in use across the world to guide clinicians in drug therapy options for pregnant women. These systems, however, lack data from human studies. Furthermore, interindividual variability in the extent of fetal drug exposure complicates the risk assessment of drug teratogenicity. In this thesis, we explored the parameters underlying this variability and we are promoting the personalization of drug therapy during pregnancy.

In **Part A**, we explored the role of placental transporter proteins in fetal drug exposure. In **chapter 2** and **chapter 3**, we determined whether the inhibition of these transporters may modulate drug exposure to the fetus. The outcome of a congenital anomaly was used as a proxy for fetal drug exposure. In these pharmacoepidemiology studies, we used population-based databases covering the Northern parts of the Netherlands: EUROCAT Northern Netherlands (NNL), a congenital anomaly registry and the IADB.nl, a pharmacy prescription database. In **chapter 2**, we focused on the role of P-glycoprotein (P-gp), a highly expressed efflux transporter in the placenta during early pregnancy. It is clinically important as one of the protective mechanisms against the possible harmful effects of a drug on the developing fetus. From the literature, we identified 105 drug substrates associated with P-gp transport. Among our population, we found that women who took certain drugs which are substrates of P-gp concurrently with another substrate or an inhibitor of P-gp, had an increased risk of having children with congenital anomalies (another substrate: OR 4.17, 95% CI 1.75–9.91; inhibitor: OR 13.03, 95% CI 3.37–50.42). Therefore, drug-drug interactions mediated by placental P-gp might modulate the level of fetal drug exposure.

In **chapter 3**, we explored the role of several placental transporters: breast cancer resistance protein, multidrug resistance-associated protein 1, organic cation transporter 3, equilibrative nucleoside transporter, organic anion transporter 4, organic anion transporting polypeptide 2B1 and monocarboxylate transporters 1, 4, 8 and 10. Based on literature findings, we have identified a list of drugs reported to be substrates, inhibitors or inducers of these transporters. However, we did not find any association between drug interactions mediated by these transporters and the risk of congenital anomalies, probably due to the low number of pregnant women who used the drugs transported by these transporters. Therefore, large-scale databases

are needed to further denote the role of these transporters in fetal drug transport in future studies.

Genetic polymorphisms of placental transporter proteins are known to modulate protein expression and/or activity. To highlight current knowledge on these polymorphisms and their effects on protein expression in the placenta, we performed a literature review in **chapter 4**. We also proposed to group these relevant genetic polymorphisms into phenotype classifications based on their effect on transporter function: 'increased', 'normal', 'decreased' and 'abolished' activities. This phenotype grouping can be used in genetic studies, and will enable us to use smaller sample sizes than the traditional candidate gene association studies.

Part B introduces the concept of pharmacogenetics and its potential use as a tool in personalization of drug therapy during pregnancy. In **chapter 5**, we performed a survey among formerly pregnant women to assess their knowledge regarding pharmacogenetics, and their attitude towards the implementation of pharmacogenetics in their future drug therapy. The study population included women who had been pregnant and had a history of medication use. The women were identified from the IADB.nl database. Nearly 70% of the respondents are aware of the relation between their genes and the response of their body to medication. Over half of the respondents (53.9%) had a positive attitude concerning the implementation of pharmacogenetics in clinical care, which is also encouraging. However, some concerns were raised, including the privacy and anonymity of genetic information, possible misuse by employers or insurance companies and a lack of understanding of the concept. We proposed that more effort should be given to educate not only the public, but also the health care providers on pharmacogenetics and what it can offer towards better drug therapy options.

The next chapters focus on the role of pharmacogenetics in drug-induced congenital anomalies, specifically on the association between the prenatal exposure to serotonin reuptake inhibitors (SRIs) and the risk of congenital heart anomalies (CHA). In **chapter 6**, we identified potential pharmacogenetic predictors relevant in the pharmacokinetics and mechanism of action of SRIs, and also possible mechanism underlying CHA. The important genetic variants in the mechanism of this purported teratogenicity include the variants in genes encoding for the metabolic enzymes (*CYPs*), P-glycoprotein (*ABCB1*), serotonin transporter (*SLC6A4*) and serotonin receptors (*HTR1A, HTR1B, HTR2A, HTR3B*).

Using the information in chapter 6, **chapter 7** describes an exploratory study on the

effect of these potential pharmacogenetic predictors on the risk of CHA among prenatally SRI-exposed children. In this case-only study, the children/fetuses with CHA were selected from the EUROCAT NNL registry. Among several variants explored, we were not able to identify any genetic variations that lead to a significant increase in the risk of CHA. The lack of association might probably be related to the limited sample size of our study. Among genetic variations potentially associated with the risk of CHA (with large confidence intervals), are the variations in P-gp (*ABCB1* rs1128503), serotonin receptors (*HTR1A* rs1364043, *HTR1B* rs6296 & rs6298, *HTR3B* rs1176744) and serotonin transporter (*SLC6A4* 5-HTTLPR & 5HTTVNTR). These predictors are part of a complex interplay between genetic variations and environmental factors contributing to CHA. In a gene-environment interaction study like this, sample sizes required to detect the effect are larger than those required to detect main genetic or environmental effects. Therefore, collaboration between registries of congenital anomalies is one of the options to achieve the required sample sizes.

Pharmacogenetics is a promising tool for clinical application during pregnancy, either in patient care or research. In future patient care, pregnant women may benefit from a tailored drug dosing and selection to ensure drug efficacy with limited fetal risks. In research, we might elucidate the mechanisms involved in drug teratology by identifying affected children by their pharmacogenetic risk factors. In line with these future prospects, we need continuous research in the areas of epidemiology and pharmacogenetics which will hopefully be valuable for preventive strategies in clinical practice.

SAMENVATTING

In veel studies naar geneesmiddelengebruik is het gebruik van geneesmiddelen tijdens de zwangerschap onderzocht en hieruit blijkt dat er een hoge prevalentie is van zwangere vrouwen die ten minste één geneesmiddel gebruiken. De tragedie rondom thalidomide in de zestiger jaren van de vorige eeuw heeft bijgedragen tot een duidelijke verbetering van de bewaking van geneesmiddelengebruik tijdens de zwangerschap. Er worden wereldwijd verschillende classificatiesystemen gebruikt om clinici te ondersteunen bij de geneesmiddelenkeuze van zwangere vrouwen. Bij deze systemen is er echter een gebrek aan data uit studies bij mensen. Bovendien wordt het vaststellen van het risico op teratogenititeit bemoeilijkt door interindividuele variabiliteit in de mate van blootstelling van de foetus aan het geneesmiddel. In dit proefschrift hebben we de parameters onderzocht die ten grondslag liggen aan deze variabiliteit en breken we een lans voor geïndividualiseerde farmacotherapie tijdens de zwangerschap.

In **Deel A**, onderzochten we de rol van transporteiwitten in de placenta bij de blootstelling van de foetus aan geneesmiddelen. In **hoofdstuk 2** en **3** bepaalden we of de remming van deze transporters de geneesmiddelenblootstelling van de foetus beïnvloeden. We hebben hierbij de uitkomst aangeboren afwijking gebruikt als surrogaat uitkomst voor geneesmiddelenblootstelling van de foetus. Voor deze farmacoepidemiologische studies hebben we twee populatie databases gebruikt die data bevatten over het noordelijke deel van Nederland: EUROCAT Noord Nederland (NNL), een registratie voor aangeboren afwijkingen en de IADB.nl, een apotheek database met informatie over afgeleverde recept geneesmiddelen. In **hoofdstuk 2** focusten we ons op de rol van het P-glycoproteïne (P-gp), een transporter met een hoge expressie in de placenta tijdens de vroege zwangerschap. Deze transporter is klinisch relevant als een beschermend mechanisme tegen mogelijk schadelijke effecten van geneesmiddelen op de zich ontwikkelende foetus. Op basis van literatuuronderzoek identificeerden we 105 geneesmiddelen die door P-gp door de placenta worden getransporteerd (P-gp substraten). In onze populatie vonden we dat vrouwen die geneesmiddelen gebruikten die worden getransporteerd door P-gp en die tegelijkertijd een ander substraat of een remmer van P-gp gebruikten, een verhoogd risico hadden op het krijgen van een kind met een aangeboren afwijking (ander substraat: OR 4,17, 95% betrouwbaarheidsinterval, BI 1,75- 9,91; remmer: OR 13,03, 95% BI 3,37- 50,42). Dit wijst erop dat interacties tussen geneesmiddelen gemedieerd door P-gp in de placenta mogelijk de mate van blootstelling van de foetus aan geneesmiddelen kan beïnvloeden.

In **hoofdstuk 3** onderzochten we de rol van diverse transporters in de placenta: breast cancer resistance eiwit, multidrug resistance eiwit 1, organic cation transporter 3, equilibrative nucleoside transporter, organic anion transporter 4, organic anion transporting polypeptide 2B1 en de monocarboxylate transporters 1, 4, 8 en 10. Op basis van literatuurgegevens, hebben we een lijst van geneesmiddelen geïdentificeerd die beschreven zijn als substraat, remmer of induceerder van deze transporters. Echter, we konden geen associatie aantonen tussen interacties van geneesmiddelen gemedieerd door deze transporters en het risico op aangeboren afwijkingen. Een mogelijke reden hiervoor is het kleine aantal zwangere vrouwen dat geneesmiddelen gebruikte die getransporteerd worden door deze transporters. Er zijn dus grotere databases nodig om de rol van deze transporters bij geneesmiddelentransport naar de foetus in toekomstige studies te onderzoeken.
Van genetische polymorfismen van transporteiwitten in de placenta is bekend dat ze de eiwitexpressie en/of activiteit kunnen beïnvloeden. We hebben in **hoofdstuk 4** een literatuuronderzoek naar deze polymorfismen en hun effect op eiwitexpressie in de placenta uitgevoerd om inzicht te krijgen in de huidige staat van kennis. We hebben ook voorgesteld om de betreffende genotypes samen te voegen in fenotypes op basis van hun effect op de functie van de transporter: toegenomen, normale, afgenomen en afwezige activiteit. Deze classificering in fenotypes kan worden toegepast in genetische studies en maakt het mogelijk om met kleinere aantallen patiënten tot uitspraken te komen dan met traditionele kandidaat-gen associatie studies het geval is.

In **Deel B** beschrijven we het concept farmacogenetica en de mogelijke toepassing hiervan bij het individualiseren van geneesmiddelengebruik (farmacotherapie) tijdens zwangerschap. In **hoofdstuk 5** hebben we een enquête uitgevoerd onder vrouwen die zwanger zijn geweest om vast te stellen hoe hun kennis over farmacogenetica is en te vragen naar hun houding ten opzichte van implementatie van farmacogenetica bij toekomstige farmacotherapie. De studie populatie bestond uit vrouwen die zwanger waren geweest en in het verleden medicijnen hadden gebruikt. Deze vrouwen hebben we geïdentificeerd via de IADB.nl database. Bijna 70% van de respondenten zijn zich bewust van de relatie tussen hun genen en de reactie van hun lichaam op geneesmiddelen. Meer dan de helft (53,9%) van de respondenten stond positief tegenover de implementatie van farmacogenetica in de klinische praktijk, hetgeen bemoedigend is. Echter er werd ook melding gemaakt van zorgen en wel omtrent privacy en anonimiteit van genetische informatie, mogelijk misbruik door werkgevers of verzekeraars en gebrek aan kennis van de farmacogenetica. Wij hebben voorgesteld om meer energie te steken in het verbreiden van kennis over farmacogenetica en wat het kan bijdragen aan betere farmacotherapie, niet alleen bij het publiek, maar ook bij zorgverleners.

De volgende hoofdstukken focussen op de rol van farmacogenetica bij door geneesmiddelen veroorzaakte aangeboren afwijkingen en wel in het bijzonder op de associatie tussen prenatale blootstelling aan serotonine heropname remmers (SRIs) en het risico op aangeboren hartafwijkingen (CHA). In hoofdstuk 6 hebben we potentiële farmacogenetische voorspellers geïdentificeerd die van belang zijn voor de farmacokinetiek en werkingsmechanismen van de SRIs en voor mogelijke mechanismen die ten grondslag liggen aan CHA. De genetische varianten van belang voor de mechanismen van deze vermoede teratogeniteit zijn te vinden in de genen voor metabole enzymen (CYPs), P-gp (*ABCB1*), serotonine transporter (*SLC6A4*) en serotonine receptoren (*HTR1A*, *HTR1B*, *HTR2A*, *HTR3B*).

Gebaseerd op de informatie van hoofdstuk 6 wordt in **hoofdstuk 7** een exploratieve studie beschreven naar het effect van deze potentiële farmacogenetische voorspellers op het risico op CHA bij kinderen die prenataal aan SRIs zijn blootgesteld. Voor deze studie hebben we gegevens van de EUROCAT NNL database gebruikt. Tussen verscheidene onderzochte voorspellers konden we geen genetische varianten identificeren die tot een significante toename van het risico op CHA leidden. Het ontbreken van een significante associatie is mogelijk het gevolg van het kleine aantal studiedeelnemers. Tot de genetische variaties die potentieel geassocieerd zijn met het risico op CHA (met grote betrouwbaarheidsintervallen) behoren de variaties in P-gp (*ABCB1* rs1128503), in drie serotinine receptoren (*HTR1A* rs1364043, *HTR1B* rs6296 & rs6298, *HTR3B* rs1176744) en in de serotonine transporter (*SLC6A4* 5-HTTLPR & 5HTTVNTR). Deze voorspellers spelen een rol in de complexe wisselwerking tussen genetische variaties en omgevingsfactoren die bijdragen aan CHA. Bij een gen-omgevingsinteractie studie zoals deze, zijn de benodigde aantallen deelnemers om een effect aan te tonen groter dan bij studies naar uitsluitend genetische of omgevingseffecten. Daarom is samenwerking tussen registraties van aangeboren afwijkingen een mogelijkheid om aan de benodigde grote aantallen te komen.

Farmacogenetica is een veelbelovend hulpmiddel voor toepassing tijdens zwangerschap, zowel voor de patiëntenzorg als voor onderzoek. Bij de patiëntenzorg van de toekomst, kunnen zwangere vrouwen mogelijk profiteren van een op het individu toegesneden dosering en geneesmiddelenkeuze om een optimale werkzaamheid en een geminimaliseerd risico voor de foetus te bereiken. Wat het onderzoek betreft kunnen we mogelijk met behulp van de farmacogenetische risicofactoren bij de kinderen de mechanismen die ten grondslag liggen aan de teratogene eigenschappen van de geneesmiddelen ophelderen. Om dit toekomstbeeld te kunnen bereiken is er behoefte aan verder onderzoek op het gebied van zowel epidemiologie als farmacogenetica om op deze wijze hopelijk te kunnen bijdragen aan preventieve strategieën in de klinische praktijk.

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LIST OF PUBLICATIONS

International publications supporting this thesis

Daud AN, Bergman JE, Bakker MK, Wang H, de Walle HEK, Plösch T, Wilffert B. Pharmacogenetics of drug-induced birth defects: the role of polymorphisms of placental transporter proteins. *Pharmacogenomics* 2014;15: 1029–1041.

Daud ANA, Bergman JEH, Bakker MK, Wang H, Kerstjens-Frederikse WS, de Walle HEK, Groen H, Bos HJ, Hak E, Wilffert B. P-Glycoprotein-Mediated Drug Interactions in Pregnancy and Changes in the Risk of Congenital Anomalies: A Case-Reference Study. *Drug Saf.* 2015;38: 651–659.

Daud ANA, Bergman JEH Kerstjens-Frederikse WS, Groen H, Wilffert B. The risk of congenital heart anomalies following prenatal exposure to serotonin reuptake inhibitors—Is pharmacogenetics the key? *Int J Mol Sci.* 2016;17: 1333.

Daud ANA, Bergman JEH, Oktora MP, Kerstjens-Frederikse WS, Groen H, Bos HJ, Hak E, Wilffert B. Maternal use of drug substrates of placental transporters and the effect of transporter-mediated drug interactions on the risk of congenital anomalies. *PLOS ONE* 2017;12(3): e0173530.

Daud ANA, Bergsma EL, Bergman JEH, De Walle HEK, Kerstjens-Frederikse WS, Bijker BJ, Hak E, Wilffert B. Knowledge and attitude regarding pharmacogenetics among formerly pregnant women in the Netherlands and their interest in pharmacogenetic research. *BMC Pregnancy Childbirth* 2017 (accepted).

Daud ANA, Bergman JEH, Kerstjens-Frederikse WS, van der Vlies P, Hak E, Berger RMF, Groen H, Wilffert B. Serotonin reuptake inhibitors and the risk of fetal congenital heart anomalies: An exploratory pharmacogenetics study (Abstract for German Pharm-Tox Summit 2017). *Naunyn-Schmiedeberg's Arch Pharmacol.* 2017;390 (Suppl 1):S24.

Conference presentations

Daud ANA, Bergman JEH, Bakker MK, Wang H, Kerstjens-Frederikse WS, de Walle HEK, Groen H, Bos HJ, Hak E, Wilffert B. P-glycoprotein-mediated drug interactions in pregnancy and the effect on the risk of congenital anomalies. Poster presentation at FIGON Dutch Medicine Days, Ede, The Netherlands, 6-8 October 2014.

Daud ANA, Bergman JEH, Bakker MK, Wang H, Kerstjens-Frederikse WS, de Walle HEK, Groen H, Bos HJ, Hak E, Wilffert B. P-glycoprotein-mediated drug interactions in pregnancy and the effect on the risk of congenital anomalies. Poster presentation at Euromedicat Conference, Poznan, Poland, 2-4 February 2015.

Daud ANA, Bergman JEH, Oktora MP, Bakker M, Kerstjens-Frederikse WS, Groen H, Bos HJ, Hak E, Wilffert B. Maternal use of drugs transported by placeental transporter proteins in the first trimester of pregnancy. Poster presentation at FIGON Dutch Medicine Days, Ede, The Netherlands, 5-7 October 2015.

Daud ANA, Bergman JEH, Kerstjens-Frederikse WS, van der Vlies P, Hak E, Berger RMF, Groen H, Wilffert B. Serotonin reuptake inhibitors and congenital heart anomalies: An exploratory pharmacogenetics study. Oral presentaton at the 18th International Conference of Genomics and Pharmacogenomics, Singapore, 8-9 January 2017.

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ABOUT THE AUTHOR

Nur Aizati Athirah Daud was born on December 27, 1985 in Kelantan, Malaysia. Her early education was in Kelantan, and she later went to Penang Matriculation College in 2003. She continued her stay in Penang for a Bachelor's degree in Pharmacy at the Universiti Sains Malaysia (2004-2008). Her first experience with research was performing a final year research project on the production of a biopolymer as a medium for drug carrier. The project was a collaboraton with Malaysian Institute of Pharmaceuticals and Nutraceuticals (IPharm). After graduating, she worked as a training pharmacist in Hospital Raja Perempuan Zainab II in Kota Bharu, Kelantan (2008-2009).

In 2010, she received a scholarship from Universiti Sains Malaysia and the Ministry of Education to further study in clinical pharmacy. She joined the MPharm programme in the School of Pharmaceutical Sciences, Universiti Sains Malaysia and graduated in 2011. Throughout this programme she had presented a series of case studies, and had performed an observational study on the side effects of lamotrigine in adult patients with epilepsy, which was presented at a national conference.

At the end of 2012, she began her PhD study at the University of Groningen, with the scholarship from the Ministry of Education Malaysia and Universiti Sains Malaysia. The topic of interest is the safety of drug use during pregnancy and personalized drug therapy during pregnancy. Throughout this period, she had presented her research projects in several national and international conferences. These projects had resulted in the publication of this thesis. Upon completion of her PhD study, she will start her career as a lecturer in the School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang.